LYMPHATICS, LYMPH AND LYMPHOID TISSUE

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LONDON

EDWARD ARNOLD (PUBLISHERS) LTD.

First edition published by Harvard University Press, 1939
Second edition published by Edward Arnold (Publishers) Ltd, 1956

Printed in Great Britain by Butler & Tanner Ltd, Frome and London

PREFACE TO THE SECOND EDITION

For the past century we have accepted the teaching of Claude Bernard that, in spite of the ever changing environment of the organism as a whole, the tissue cells are always surrounded by a fluid of constant properties. Together with the blood plasma this medium forms the extracellular fluid; and, although we can readily determine the composition of the circulating plasma, no one has yet succeeded in collecting and analysing the fluid which forms the immediate environment of the cells in a normal animal.

It is a little less than a century ago since Ludwig and his pupils first collected lymph from lymphatic vessels in different parts of the body. Lymph is tissue fluid which arises from the blood by passing through the endothelium of the blood capillary, undergoes varying degrees of modification in the tissues, then traverses the endothelium of the lymphatic capillary. There has been much controversy about the precise relationship between lymph, and the interstitial fluid from which it arises; we believe now that there are only minor differences between these fluids. But be that as it may, the formation of lymph must be related to the movement of fluid across capillary membranes, and in its study we have therefore learned much about the intricacies of capillary structure and the meaning of capillary permeability.

The functions of lymph and of the lymphatic system have been of interest ever since Asellius in 1627 first discovered the lacteals, but more so since Ludwig showed that lymph could be collected and analysed We believe that the main purpose of the lymphatics is to return to the blood stream the large protein molecules which slowly, but continuously leak through the pores of the capillaries The rôle of the proteins in maintaining the volume of the circulating plasma, and the function of the lymphatic vessels in returning extravascular protein to the blood stream, form the thesis which repeatedly recurs throughout this monograph.

Closely associated with the lymph and blood streams we find, most characteristically in mammals, the tissues which we designate as "lymphoid". These are composed of several different kinds of cells which are intermingled in varying proportions, and in which the most numerous elements are lymphocytes. For many years the lymphoid tissues have been of interest mainly because of the changes they undergo in pathological conditions.

Though we have by no means disregarded pathological changes, to the understanding of which many interesting contributions have been

made in recent years, we have devoted our attention for the most part to the problems of lymphoid tissue in health. In such tissue there is a dynamic equilibrium maintained, by means as yet unknown, between the component cell types. It is the combination of these cells in varying proportions which give the different lymphoid tissues their distinctive characters We do not as yet understand how these cellular equilibria are preserved, and for the most part we can merely record disturbances as they occur.

The function which is most clearly established for normal lymphoid tissue is the continuous production of lymphocytes, and we have endeavoured as far as possible to approach this from a quantitative point of view. We have tried also to use the same kind of approach in the case of the bone marrow, in an effort to elucidate the relationship between the two major components of the haemopoietic system

The first edition of this book appeared in 1940. Since that time there have been numerous advances in our knowledge of the lymphatic system, and a second edition has long been overdue. Many of the advances which have been made owe their inspiration, directly or indirectly, to the now classical work of Dr. C. K Drinker and his collaborators It is therefore with profound regret that we learned, shortly before this volume appeared, of Dr. Drinker's untimely death

There is by now little need to eulogize the many fundamental contributions which have been made by Dr. Drinker to our knowledge of the lymphatic system One of us, however (J M. Y.), would like to place on record his great sense of personal indebtedness to the late Dr Drinker for the opportunity of working with him for two years at Harvard, as a Fellow of the Rockefeller Foundation, immediately before the second world war

We would also like to express our appreciation of the great generosity, both of the late Dr. Drinker and of the Harvard University Press, in permitting us to draw freely and without reservation on the subject-matter of the first edition We need hardly add, however, that for the views and statements appearing in the present volume we alone are responsible. We are also grateful to the Editor of the Cambridge Biological Reviews for permission to quote freely from a review published by one of us (J. M. Y.) in 1950.

In the present edition Dr. Drinker's place has been taken by Courtice, who has been responsible for Chapters II, III, IV and VIII, while Yoffey has been responsible for Chapters V, VI and VII. Chapter I, as before, has been written jointly.

As with all monographs of this nature, the besetting problem is that of compression and condensation within reasonable bounds. The present edition is some 20 per cent larger than the first. It could readily have

been expanded to twice its size, but to do so would have defeated one of its main objects, namely to make it sufficiently readable to arouse interest among many whose field of work was not too highly specialized. We have deliberately condensed a number of sections which could with advantage have undergone considerable expansion. In our endeavour to make the book shorter and more readable, we have inevitably refrained from mentioning many papers which were undoubtedly worthy of inclusion, and we have therefore tried to refer in many instances to review papers and monographs which would give access to a fuller bibliography. We trust that those of our colleagues who find themselves confronted with similar problems will sympathize with us and forgive our many sins of omission.

It gives us great pleasure to place on record our indebtedness to Miss L. Lloyd and Miss M. J Poole, for their invaluable assistance in the preparation of the manuscript; to Miss J. A. Ashby and Mr A. E. S. Roberts for their indefatigable bibliographic efforts on our behalf, to Mr. Graham Frankcom who has redrawn a number of figures for this edition, and Mr. R. Money and Mr M. H. G Maggs who have prepared many of the photographs.

We should also like to acknowledge our gratitude to the following for their kind permission to reproduce figures. Professor Sir Wilfrid Le Gros Clark, F.R.S., and the Oxford University Press (*The Tissues of the Body*); Dr. Robert B. Rowan, Managing Editor of the W B Saunders Company, Dr. William Bloom (*A Text-Book of Histology* by Maximow and Bloom); Dr W B. Arey (*Developmental Anatomy*), Professor J C. Brash and the Oxford University Press (Cunningham's Text-Book of Anatomy), Professor V. Menkin and the MacMillan Company (*Dynamics of Inflammation*); The Harvard University Press (*Pulmonary Oedema and Inflammation*); Charles C. Thomas (*The Lung*); Gustav Fischer (*Das Lymphgefässsystem*); The Editors of *Advances in Internal Medicine, American Journal of Anatomy, American Journal of Physiology, American Scientist, Anatomical Record, Annals of Surgery, Annals of the Royal College of Surgeons, Archives of Diseases in Childhood, Australian Journal of Experimental Biology and Medical Science, Biological Reviews, British Journal of Experimental Pathology, Johns Hopkins Hospital Reports, Journal of Anatomy, Journal of Cellular and Comparative Physiology, Journal of Clinical Investigation, Journal of Experimental Medicine, Journal of Pathology and Bacteriology, Journal of Physiology, Physiological Reviews, Quarterly Journal of Experimental Physiology, Surgery* and the *Harvey Lectures*, and to the several authors of the figures used from these journals, to Professor W. O. Reinhardt for the [illegible] 83, to Mr Bede [illegible] 154; to Dr. W. J. [illegible], II, III, IV and

VIII and making many helpful comments and suggestions; and to many others too numerous to mention who have made this book possible

J. M. YOFFEY
F. C. COURTICE

Bristol and Sydney
August 1956

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CHAPTER I

GENERAL ARRANGEMENT AND ORGANIZATION OF THE LYMPHATIC SYSTEM

Introduction

This monograph is an effort to set forth some of the salient features of the organization and function of the lymphatic apparatus in mammals and to indicate where possible the clinical significance of the data thus presented. The lymphatic system, as we view its gradual development from fish to mammals, shows a steady increase in complexity and organization, an increase which is an expression of the fundamental importance to mammals of the lymphatic apparatus. Yet in spite of the great advance in medical science made during recent years, there are still very serious gaps in our knowledge of the ultimate functions of lymphatics, lymphocytes and the organized collections of lymphocytes which we term lymphoid tissue. We know that if lymphocytes and lymphoid tissue suffer extensive destruction, death occurs; and that if lymphatics are blocked, disastrous changes appear in the regions involved; but the real reasons behind either of these gross results still elude us. To solve such puzzles, it is essential, first of all, to approach the lymphatic apparatus from what may be termed the standpoint of anatomical physiology, to describe the elaborate system of lymphatic vessels, the lymphocytes and the collections of these cells, both unorganized and organized; and to assign to these various elements in our problem the physiological and medical values known to us today.

Historical Observations

The thin-walled lymphatic vessels, collapsed after death, escaped attention for many centuries after the blood vessels had first been described. Even now that their existence is clearly recognized, it is doubtful whether more than a very small number of those who are concerned with the lymphatic system ever actually see for themselves either lymph vessels or lymph. Though lymphatics had been seen by members of the Alexandrian school (Herophilos, 300 B.C.; Erasistratus, 310–250 B.C.), the modern study of the lymphatics dates from 1622, when Gasparo Aselli, professor of anatomy and surgery in Pavia, a city near Milan, described the appearance of lymphatic vessels in the mesentery of a well-fed dog. Aselli not only observed distended lacteals in the mesentery, but on pricking one of them saw "a white liquid milk or

lymphatic system has become comparatively isolated from the blood vascular system.

In such creatures as jellyfish there is no blood circulation in the sense with which we are concerned. The internal environment of their cells is sea water—identical with the external environment. As we progress upward in the phylogenetic scale and the need for greater activity develops, tubular systems carrying a pigment capable of transporting oxygen appear. A heart, of increasing complexity, becomes important and the circulating medium begins to contain increasing amounts of protein.

With such arrangements—arrangements which provide a different environment for the cells of the body from that which surrounds it on the outside—it apparently becomes necessary to provide a mechanism other than the blood vascular system for clearing the tissue of substances not readily absorbed by the blood. Thus, in certain fish one sees vessels —venous lymphatics—which contain blood and lymph in varying proportions; and with these vessels, lymph hearts appear. When lymph hearts are present, the lymph is propelled back to the circulation in an active manner, and does not depend for forward movement upon accidents of motion, massage, etc., as in the mammal. No valves are present in the lymphatics of fish, amphibia and reptiles; these creatures have functional lymph hearts which effect a sluggish but direct flow of lymph to the blood.

In most birds the lymph hearts disappear after embryonic life and the lymph vessels contain valves, just as do those of mammals. Lymph nodes are present in but few species of birds and are of comparatively simple character. The lymphatic apparatus—vessels, nodes, etc.—reaches its highest complexity of structure and function in mammals.

Lymphatic Vessels

Function of Lymphatic Vessels

The lymphatic vessels in the mammal form a fine network of thin-walled capillaries which drain into larger and thicker-walled collecting trunks and ultimately join with the great veins at the base of the neck. At the periphery, intercommunication between the smaller vessels is so free as to make it extremely difficult to destroy all lymph movement in a given region The lymph which these vessels contain originates from the blood stream by filtration through the capillary membrane and is on its way back to the blood. Thus there is formed within the extra-cellular phase of the body a circulation which is slow compared with the intravascular, but nevertheless an essential process in the normal life of the cells. There seems no doubt that the lymphatic system has

cream forthwith gush out". As a true disciple of Galen, he believed that these vessels went to the liver, where their contents were "concocted" into blood. However, in 1651 Jean Pecquet in France described the receptaculum chyli and the thoracic duct, through which the "liquor of the Milkie Veins" finally "throws itself headlong into the *Whirlpool of the Heart*". The term "lymphatics" was first used by Thomas Bartholin (1653).

By the time of William Hunter (1718–1783) much was known about the gross anatomy of the lymphatic system in man and other mammals, and Hunter believed that "the lymphatic vessels are the absorbing vessels, all over the body". Though this generalization is not too wide of the mark, the evidence for it could not be obtained until a good deal more was known about the structure and properties of the blood capillary, first described by Malpighi in 1661. William Hewson, a pupil whom William Hunter "bred to anatomy", made extensive dissections of the lymphatic system in fishes, birds and mammals. He noted that lymph glands were absent in fishes (also in the turtle), few in number in birds and well developed only in mammals. He also noted the presence of lymphocytes in lymph and thought they came from the lymph glands to enter the blood via the lymph channels.

A new chapter in the study of the lymphatic system may be said to have commenced with Karl Ludwig (1816–1895), who developed techniques for the collection of lymph from lymphatic vessels in different parts of the body Ludwig thought that lymph was a filtrate derived from blood and, after one of the classical controversies of physiology, this concept was finally established on a firm foundation by Ernest Starling (1866–1927), who was the first to point out the fundamental relationship between the hydrostatic pressure of the blood in the capillaries and the colloid osmotic pressure of the plasma proteins.

We cannot do better here than quote the assessment of Starling's work given by Drinker (1942) "Those who have re-worked the field, though able to describe many things which would have been immensely interesting and gratifying to Starling, have added comparatively little to the fundamental principles which he formulated of lymph formation and of the movement of fluid from and into blood capillaries. Starling's evidence, though often technically crude, was, nevertheless, the product of an intelligence which had a habit of being right in the great essentials."

Evolution

To attempt to describe the evolution of the circulation of the blood as we see it in mammals would require far more than the scope of this book. It will, however, be worth while to trace briefly the steps by which the

grow the ilio-lumbar plexus, the femoral plexus and the plexus surrounding the umbilical artery. The cisterna chyli and the lower part of the thoracic duct arise in common with the iliac sacs from the mesonephritic veins. The thoracic duct is formed by the union of a duct which grows downward from the left jugular sac and a plexus arising from the cisterna chyli. The retroperitoneal sac gives rise to the lymphatics of the abdominal viscera (Heuer, 1909). All of the abdominal sacs, the retroperitoneal ventral to the aorta, the two lateral iliac sacs and the cisterna chyli dorsal to the aorta connect with each other.

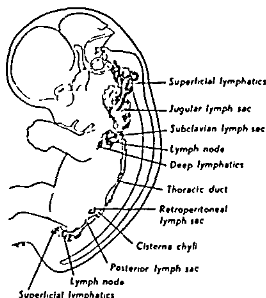


FIG 1 —Reconstruction of the primitive lymphatic system showing the primary lymph sacs in a human embryo of two months, after Sabin
(From Arey, *Textbook of Developmental Anatomy*, W B Saunders Co Philadelphia)

The primary lymph sacs do not persist as such. In mammals they ultimately become primary lymph nodes, while the secondary nodes develop along the course of the lymph ducts. In other species, however, [illegible]

while in amphibia they reach their highest development. The primary lymph sacs which are destined to become lymph hearts bud off from segmental veins and rest on the myotomes from which they derive muscle fibres. The anterior lymph sac in birds and all the lymph sacs in mammals, on the other hand, arise from other veins and are not converted to

developed by physiological necessity to remove from the tissue fluid substances which cannot readily gain entry to the blood capillaries. Under normal circumstances these substances are mainly the plasma proteins.

The more complex and active the organism, the greater the need of the tissues for oxygen and nutritive substances in solution. This necessitates a rise in blood pressure, and with it an increase in the size and functional intricacy of the heart. A rise in blood pressure *per se* would greatly increase the escape of fluid from the capillaries, but this is prevented by a corresponding increase in the plasma proteins, whose osmotic action opposes the capillary blood pressure. Although these large protein molecules do not readily pass through the capillary membrane, in the course of a day something like 50 per cent or more of the total circulating protein escapes from the blood vessels. This extravascular protein cannot be directly reabsorbed into the blood capillaries, but readily enters the lymphatic vessels and is returned to the blood indirectly.

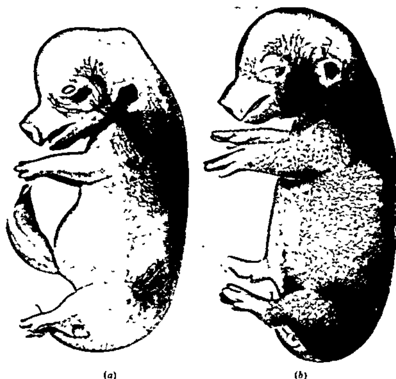
The function of the lymphatic vessels is, therefore, one of absorption from the interstitial spaces, and this function is of fundamental importance, not only in the normal maintenance of fluid balance by restoring lost protein to the circulation, but also in the response of the organism to infection and in the spread of disease from one part of the body to another. Before considering in more detail the physiological significance of the lymphatic vessels, a brief outline of the development and of the anatomy of these vessels seems necessary for a clearer understanding of function.

Origin and Development of Lymphatic Vessels

Although there has been much controversy concerning the origin of the lymphatic vessels, it now seems fairly certain that in the embryo the primary lymph sacs bud off from the veins (Sabin, 1902, 1904, 1911, 1916; Lewis, 1906, Huntington and McClure, 1908; Huntington, 1911). In the mammal two sets of paired sacs, jugular and iliac, and two unpaired sacs, retroperitoneal and cisterna chyli, are formed (Fig. 1). The jugular sacs bud off from the anterior cardinal veins and form large sacs in the neck. All the other lymphatic sacs bud off from the mesonephritic vein and the veins in the dorsomedial edge of the Wolffian bodies.

From these primary lymphatic sacs which are in communication with the veins, the peripheral lymphatic system grows by a process of endothelial sprouting. The jugular lymph sacs give rise to the lymphatics of the head and neck, forelegs, thorax, heart and lungs. The iliac sacs are two long symmetrical sacs extending from the hilus of the Wolffian bodies to the level of the bifurcation of the aorta; from the caudal end

of study, viz. (1) the injection method (2) the direct observation of growing lymphatics either in the tail of the frog larva, or in mammalian tissue in the transparent rabbit-ear chamber developed by Sandison (1924). Using the injection method in the pig embryo, Sabin (1904) showed that, starting from the region of each of the primitive sacs, there is in the skin



(a) (b)

FIG 2 —The growth of the lymphatic vessels of the embryo of the pig depicted by the injection method of Sabin (1904)

(a) is an embryo 4 3 cm. long
(b) is an embryo 5 5 cm long

The origin and centrifugal growth of the peripheral lymphatics from the primary lymph sacs are clearly shown

a gradually increasing zone of injectable lymphatics which eventually covers the whole body (Fig. 2). This injection method did not show up any isolated lymphatics, which suggests that growth is towards the periphery by the sprouting of pre-existing endothelium.

Observations in living tissues have shown that this view is no longer in doubt. Many early workers used the tail of the frog larva to study the growth of the lymphatics (cf. Koelliker, 1846; Remak, 1850; Langer,

lymph hearts but to primary lymph glands. The lymph hearts act as contractile reservoirs, receiving the centripetal flow of lymph from the general systemic and peripheral lymphatics. By virtue of their muscular contractility they are able to empty the lymphatic fluid accumulated in their cavity into the venous system, and as a secondary function it is possible that they promote the centripetal flow of lymph from the periphery by a pumping or suction action (Huntington, 1908).

The lymph hearts of the amphibia must not be confused with the subcutaneous and deep lymph sacs which have been used by physiologists to obtain samples of lymph and for the injection of drugs. These lymph sacs are not primary, but secondary, being transformed lymphatic ducts (Knower, 1908)

We see therefore that the primary lymph sacs, which bud off from the veins in the embryo, become lymph hearts in amphibia, lymph heart and lymph gland in birds, and lymph glands in mammals. The peripheral vessels grow out from these primary sacs to form lymph ducts and lymph capillaries. In amphibia the lymph ducts form large secondary lymph sacs, whereas in the mammal secondary lymph glands develop along the course of the ducts.

Growth of Lymphatic Vessels

In the original development of the lymphatic system and in any subsequent growth of lymphatic vessels, the endothelium is the essential tissue. Lymphatic endothelium arises in the first instance from the venous endothelium, but it then grows as a specific tissue.

Although it is now generally agreed that the primary lymph sacs arise by budding of the venous endothelium, there has been much contradictory experimental evidence concerning the formation of the peripheral lymphatics. Huntington and McClure (1906) and Huntington (1911), among others, maintained that lymph spaces were first formed in the tissues; these acquired an endothelium by the differentiation of mesenchyme cells, and by centripetal growth ultimately joined with the primary sacs. This view depends largely upon the results of studies of serial sections with graphic wax-plate reconstructions and has recently been supported by Balankura (1951). Clark (1911) doubts, however, whether any study of the growing lymphatic in serial sections is perfectly reliable, because all the lymphatic endothelium present at any period of development cannot be seen in stained cross-section, nor can the lymphatics always be differentiated from blood vessels, perineural spaces and shrinkage spaces.

The evidence for the view now generally held, that the lymphatic endothelium is specific and that growth of the lymphatic vessels is by endothelial sprouting in a centrifugal direction, is based on two methods

they observed that the lymphatic capillary wall was of delicate endothelium in which the nuclear thickenings stood out distinctly as clear lens-shaped structures, or as large rounded swellings which bulge into the lumen. When the same lymphatic capillary was watched for some time, a short, solid protoplasmic thread was observed to extend from the vessel. In the course of hours this increased in length and a lumen extended into a new sprout. As this sprout advanced, nuclear areas moved along the endothelial wall and new nuclei were formed by mitotic

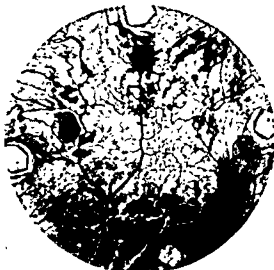


FIG. 4.—Photomicrograph showing rich lymphatic plexus in ear-chamber preparation in the rabbit.

Blood vessels first invaded the table area six days after operation and anastomosed across the centre a twenty-four days. Lymphatics first started to grow in at nineteen days and the sending out of new lymphatic sprouts continued for several months. The beaded appearance of lymphatics is well shown. In the upper part of the photomicrograph developing fat is seen.

(From Clark and Clark, 1932)

division. The growth of the mammalian lymphatic thus appears to resemble that of the vessels in the tadpole's tail.

These growth processes can also apparently be reversed. Many of the newly formed capillaries may retract or disappear, in some instances by a process which is the opposite of sprouting, in others by what appears to be a dissolution of the endothelium.

In both the mammal and the amphibian the growth of the lymphatics is always secondary to that of the blood capillaries. In the rabbit's ear, for example, in one group of experiments the blood vessels invaded the observation area in an average time of seven days, whereas the lymphatic vessels had not grown into the same area until nineteen days. In their

1868; Rouget, 1873) Clark (1909), returning to this method of investigation, observed that from the walls of the lymphatics fine pointed projections extend at various intervals and of varying lengths, while the tip ends in one or more pointed processes. This living tip is always changing and the nuclear thickenings in the wall of the capillary are perpetually altering shape and position (Fig 3) One of the processes may increase

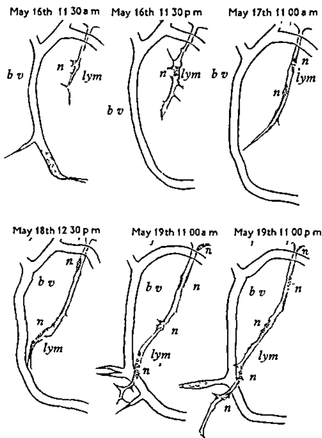


FIG 3—Successive stages in the growth of a lymphatic vessel in the frog larva
b v = blood vessel, *lym* = lymphatic, *n* = nuclear thickening
(From Clark, 1909)

in size and the lumen will then follow. Further nuclear thickenings arise by division of pre-existing ones and they move on into the lengthening wall When several of the growing tips from neighbouring capillaries anastomose, a lymphatic plexus is formed.

In the mammal Clark and Clark (1932, 1933, 1937, 1938) have used the ear chamber technique in the rabbit to observe the lymphatic vessels growing into the observation area (Fig. 4). Using high magnification

a muscular coat and an outer adventitial coat. But often it would perhaps be better to regard the lymph ducts as composed of a continuous endothelial layer, covered by a diffuse connective tissue sheath in which elastic and muscular elements are irregularly scattered. In those lymphatic vessels which contain smooth muscle in their walls, amyelinated nerves can be traced to the muscle fibres, suggesting that [illegible] e motor in function.

A prom[illegible] s (Fig. [illegible] appear [illegible] of graphite. In [illegible] lymphatic vessels the



FIG 6.—Photomicrographs of fully formed valves, one sectioned transversely, *A*, and another longitudinally, *B* The flaps of the valves are in continuity with each other at the buttress, *p*

(From Kampmeier, 1928)

primary or superficial plexus of the skin covering the body contains no valves (Sabin, 1904) and the whole lymphatic network can easily be injected The most active generation of valves occurs between the end of the second and the beginning of the fifth month of foetal life in man (Kampmeier, 1927–1928). At the end of the second month some valves are forming at the points at which lymph channels are confluent with the jugular lymph sac. In the three-month embryo, rudiments of valves appear in the thoracic duct and in the lymphatics of the leg. In the next month they appear throughout the thoracic duct and throughout the

growth lymphatic capillaries sometimes lie adjacent to venules or blood capillaries with nothing between the walls of the two vessels, but they never join with blood capillaries (Clark and Clark, 1937). Even when lymphatic capillaries become isolated by injury, they may ultimately reach and anastomose with other lymphatic vessels, but never with blood capillaries. It seems improbable, therefore, that there are ever any communications between the small lymphatic and blood vessels.

Structure of Lymphatic Vessels

von Recklinghausen (1862) first showed by staining with silver nitrate that the lymphatic capillaries are lined by a definite thin layer of flat polygonal endothelial cells whose margins stain black with silver (Fig. 5), but he also concluded that the lymphatic capillaries communicated by means of canaliculi with the tissue spaces By using the injection technique, however, Ranvier (1897), MacCallum (1903), Sabin (1904) and later Clark (1909), Clark and Clark (1932, 1937) and Pfuhl (1939,

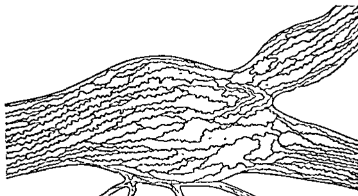


FIG 5 —Small lymphatic vessels stained with silver nitrate to show the outline of the endothelial cells

(From Bartels, 1909)

1940) concluded that the lymphatic capillaries formed a closed system and were not in direct communication with the interstitial spaces. These lymphatic capillaries consist solely of endothelium, which also forms a continuous lining throughout the system. With the spread of the lymphatic vessels some of these capillaries enlarge and acquire an adventitial layer to become collecting ducts. The thicker-walled collecting ducts are usually situated more deeply than the capillary plexuses. In the mesentery, Carleton and Florey (1927) showed that medium-sized vessels, 100–200 μ in diameter, contain muscle fibres, but in vessels smaller than this no muscle was observed. The walls of the large lymphatic trunks are described as consisting of three layers, the endothelium,

the liver, the ultimate functional unit, the lobule, is not supplied with lymphatic capillaries. The fluid leaving the liver sinusoids passes through capillary endothelium, and in the lobule lies between this endothelium and the liver cells. At the periphery of the lobule lymphatic capillaries are found and these carry the highly proteinized liver lymph to collecting trunks which join the thoracic and right lymph ducts. In the spleen, too, lymphatics are observed only in the capsule and the thickest trabeculae. Fluid which filters through the walls of the capillaries and sinuses must permeate the stroma before reaching the lymphatic vessels. On the other hand tissues near the surface of the body are richly supplied with lymphatic vessels. Such tissues are the skin and the mucous membranes of the gastro-intestinal and respiratory tracts.

General Anatomical Arrangement of the Main Collecting Trunks

The lymphatic trunks join the subclavian or jugular veins near their junction, where the jugular lymph sacs were originally formed in the embryo (Fig. 7). On the left side the deep cervical duct draining the head and neck, the subclavian duct draining the arm or foreleg, and the thoracic duct draining the abdominal viscera and lower extremities enter the venous system in close association with one another; but the precise details of their arrangement are subject to considerable variation. Sometimes they all empty into a sinus or dilatation which then enters the vein, at other times they may form a network before entering the vein, or they may all enter the vein close together but independently of one another. On the right side, the deep cervical duct, the subclavian duct and the right lymph duct enter the veins, and here again the arrangement may be very variable. This variability in the arrangement of the right lymph duct makes it often difficult or impossible to cannulate. Sometimes it crosses over to the left side, and at others it consists of many small ducts into which a cannula cannot be inserted

To the physiologist it is often important to collect all the lymph from the thoracic or right lymph duct, or to exclude all the lymph from the circulation. The existence of anastomoses between the main lymphatic trunks, or of additional entrances into the veins, must obviously have an important bearing on the possibility of such experimental procedures Lee (1922) ligated the thoracic duct of the cat and found that in some cases a collateral lymph circulation was established with the right lymph duct. In other instances lymphatico-venous connexions were found to exist between thoracic duct and azygos vein. Freeman (1942) made a careful study of the thoracic duct in 25 dogs to elucidate its mode of entrance into the veins. He found no branches in 3 dogs, divided left ducts in 5, branches to right side in 8, branches to azygos vein in 5, and branches

entire length of the leg in large numbers. Thus, in the embryo, the first valves appear in the developing net in the territory of the jugular lymph sacs and in the upper segment of the thoracic duct; this is followed by the development of valves in the plexuses of the extremities, and lastly throughout the entire thoracic duct itself.

Kampmeier described three distinct types of valve formation. The first type occurs when one lymph vessel joins another in the formation of a lymphatic plexus, the second type develops at the mouths of anastomoses already formed, while the third appears in the course of the principal lymphatic duct long after its continuity has been established. These lymphatic valves are usually unicuspid or bicuspid, not tricuspid. Their significance lies in the determination of the direction of lymph flow when the vessels are compressed by muscular contraction.

Distribution of Lymphatic Vessels

Although the lymphatic capillaries spread into a tissue after the blood vessels, the density of the lymphatic plexus does not always run parallel with the richness of blood supply. In the central nervous system there is no invasion by lymphatics, so that in the adult organism there are no lymphatic vessels in this tissue (cf. Sabin, 1916). No one has described lymphatics in bone marrow. Drinker, Drinker and Lund (1922) attempted to analyse the physiological possibilities of a circulation enclosed in a bony case, such as that of the marrow, in which the circulating blood may vary in volume. In the marrow, water and dissolved substances must reach the tissues very freely. Indeed, the one point settled by the paper cited is the fact that the marrow capillaries do not communicate directly with the marrow stroma, but are extraordinarily delicate membranous structures—so much so that their walls escape observation if not outlined by an injection mass. There seems no doubt that water and solutes leave the marrow capillaries and enter the marrow pulp. The arteries of the bone marrow are plentifully supplied with vasoconstrictor nerves (Drinker and Drinker, 1916) It seems reasonable to suppose therefore that if, over a period of time, the marrow were rendered turgid by leakage from capillaries subjected to vigorous blood flow, an ensuing slowing of capillary flow and fall in pressure would result in restoration of fluid and solutes to the blood stream.

Voluntary muscle, which forms the bulk of the mammalian tissue and which is richly supplied with blood vessels, contains lymphatics only in the fascial planes. Muscular contraction is thought to squeeze interstitial fluid into these lymphatics. It is generally believed that lymphatic capillaries do not actually reach the pulmonary alveoli, but that their distribution ceases at the beginning of the respiratory portion of the ultimate lung structure, the atrium leading into the alveolus. Similarly, in

the blood stream, and these authors interpreted this as indicating the existence of accessory lymphatico-venous connexions. Drinker and his colleagues have also described communications between the thoracic and right lymph ducts of dogs (cf. Drinker, 1945).

It has been our experience that, in the majority of instances, the lymph from the right lymph duct of cats and of dogs is perfectly clear. In some cases, however, one or more branches of the thoracic duct have been observed to cross to the right side, and then the lymph collected from the right lymph duct is milky through admixture with chyle. We have also observed that while the lymph from the right lymph duct may be clear during quiet breathing, it sometimes becomes quite milky when the breathing is stimulated with 5 per cent carbon dioxide (Courtice and Steinbeck, 1950). These findings suggest that between the right lymph duct and the thoracic duct there may be intercommunications which function only when the flow or pressure of lymph are substantially increased Carlsten and Olin (1952), on the other hand, failed to find any indication of such anastomoses on X-ray examination after the injection of radio-opaque substances into a mesenteric lymphatic of cats

The evidence therefore suggests that in most cats and dogs in which lymph is flowing freely, the thoracic duct empties the greater part of its contents into the great veins at the base of the neck on the left side. In some instances, however, there are quite considerable anastomoses with the right lymph duct. In addition to these major channels, there may be smaller accessory communications, either with the right lymph duct or with other veins, along which there normally passes only little or no lymph, but which become enlarged when the main channels are obstructed or the lymph flow and pressure are greatly increased

In other animals these accessory communications may be more important. Job (1918) concludes that in the common rat, there are additional communications either with the posterior part of the inferior vena cava or with the renal or portal vein region in 40 per cent of cases, while Silvester (1911–1912) has shown that in New World monkeys the main openings of the mesenteric and lower limb lymphatics are into the inferior vena cava near the renal veins.

Cannulation of Lymphatic Vessels

The physiologist is also often interested in collecting lymph from an organ or region in order to determine its rate of flow and composition. The larger collecting ducts in several parts of the body can be cannulated with a glass cannula or by polyethylene or some such plastic tubing (Fig 8) When a glass cannula is used, the lymph will soon clot and block the tip unless special precautions are taken Usually dry heparin on a loop of fine wire is periodically inserted into the cannula so that the

to right side and to azygos vein in 4. In a further group of 12 dogs killed at periods up to 225 days after tying the thoracic duct at the base of the neck, all showed various types of lymphatico-venous communications which were probably enlargements of pathways normally present. Blalock *et al.* (1937) also found evidence of the enlargement of accessory

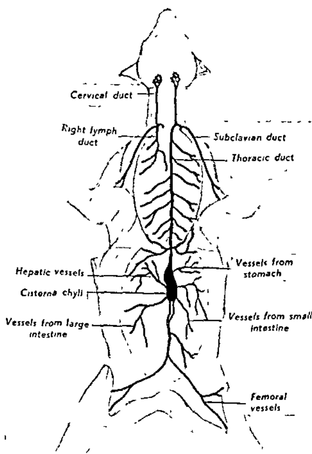


FIG 7.—Diagrammatic representation of the main collecting channels.

entrances into veins, or of anastomotic channels with the right lymph duct in the majority of, but not all, animals after ligature of the thoracic duct. It is improbable that fresh entrances into veins will develop *de novo* in these circumstances It is more likely that there is enlargement of pre-existing subsidiary channels which normally carry little or no lymph. Glenn *et al.* (1949) cannulated the thoracic duct and injected the dye T1824 centrally in a leg lymphatic. Some of the dye always entered

the blood stream, and these authors interpreted this as indicating the existence of accessory lymphatico-venous connexions. Drinker and his colleagues have also described communications between the thoracic and right lymph ducts of dogs (cf. Drinker, 1945).

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(*a*)



(*b*)

FIG 8—(*a*) The thoracic duct of a cat (head at lower end of photograph) cannulated with a polyethylene tube and the left cervical duct cannulated with a glass cannula (*b*) A close-up of the cannulae in place

Note the milky lymph coming from the thoracic duct and the clear lymph in the cannula in the cervical duct In the glass cannula is a wire with a loop on the end, by means of which powdered heparin is from time to time introduced to prevent clotting (By courtesy of Mr Bede Morris)

heparin slowly dissolves near the tip. Until recent years it was not possible to produce in animals a lymphatic fistula which would continue to flow for days. When polyethylene or transflex tubing is used, however, clotting does not occur so readily and lymph may be collected continuously over a period of several days in unanaesthetized animals (Bollman, Cain and Grindlay, 1948; Grindlay *et al.*, 1950; Brown and Hardenbergh, 1951; Tasker, 1951). Though these tubes do sometimes become blocked they may often be unblocked by the use of a very fine wire to catch and withdraw the clot.

Often, one of the main difficulties in cannulating lymphatic ducts is to find them. If they cannot readily be identified, it is best to inject a vital dye such as T1824 into the organ or region drained by the lymphatic which is to be cannulated. The dye should rapidly pass into the lymphatics and show up the collecting trunks. Having found the lymphatic vessel, the portion into which the cannula is to be inserted has to be cleaned of connective tissue sufficiently to enable the vessel to be incised and a cannula inserted. If the vessel is cleaned too thoroughly, however, it is likely to contract down to a fine thread which makes it impossible to cannulate. Only experience will guide the operator in assessing the degree of isolation from the supporting tissues and the amount of mechanical handling that a lymphatic vessel will tolerate without contracting too much.

It is always easier to cannulate a lymphatic vessel which has a good flow. In general these lymphatics will be found in young and not fully developed animals rather than in large, old animals in which the tissues may be either very fatty, or else fibrous and tough. In these latter cases the lymphatics may be more difficult to find and dissect, and the flow may be small with thread-like vessels. Again it does not follow that the larger the animal, the bigger the lymphatics and the easier to cannulate. Another obstacle to ease of cannulation may be the closely arranged valves. Once the cannula is tied in place a valve blocking the flow may be broken down by passing a fine wire down the cannula into the vessel.

Lymph flow will be dealt with in Chapter 4, but it should be emphasized here that with certain exceptions, once a lymph trunk is cannulated lymph will flow spontaneously only if there is passive or active movement of the part, or if the tissue tension is greatly increased above normal as in an acute inflammatory reaction. In the quiescent limb of an anaesthetized animal there will be no spontaneous lymph flow, but lymph may be collected in the cannula by passive movement, or by massage of the limb from the periphery towards the cannula along the line of the duct. The flow from the right lymph duct and from the thoracic duct on the other hand will be spontaneous, influenced in varying degrees by respiratory movement.

Regenerative Powers of Lymphatic Vessels

In mechanical injury where the lymphatic vessels have been severed, and in inflammation due to various causes, the lymphatic vessels have a remarkable power of regeneration. Clark (1922–1923) made a cut in one of the tail fins of the frog larva and observed in the following days that the cut lymphatics ultimately joined up by sending out threads of protoplasm, as do normally growing capillaries. When lymphatic capillaries are isolated experimentally they retain their specificity and their power of growth for periods up to 3 weeks or more and may eventually re-establish continuity with the lymphatic system. In mammalian tissues, Reichert (1926) observed the regeneration of the lymphatic vessels in the dog after complete transection of all the tissues of the thigh (with the exception of the femur, femoral artery and vein) and subsequent approximation of the edges. The superficial set of lymphatics in the skin was on occasion observed to show some regeneration as early as 4 days after the operation, while the deep set of lymphatics began to regenerate from 8 days after severance. This bridging of the wound by regenerating lymphatics occurred at sites where there was least reaction and scarring. Where there was appreciable scarring or where healing had been interrupted by a foreign body or infection, the growth of lymphatics was delayed for weeks or months. In time the main lymphatic trunks also reunited. *On the other hand Meyer (1906) found no regeneration after* ligating and resecting 3 to 5 mm of the large lymphatic trunks in the leg of the dog.

McMaster and Hudack (1934) found that injection of dye showed up many small channels, within 7 to 10 days, in and about incisions made in the skin of the mouse's ear. They also observed proliferation of the lymphatic vessels around the site of a thermal burn, while Pullinger and Florey (1937) observed intense proliferation of lymphatic capillaries in the ear of the mouse following inflammatory reactions due to tar and silica (Fig. 9). In any traumatic or other inflammatory reaction, the regeneration and proliferation of the lymphatic vessels is just as important as that of the blood vessels. When the reaction resolves, the lymphatic vessels retrogress

The purpose of this proliferation is no doubt to absorb the excess of proteins and other particles which may be present in the interstitial spaces as a result of the inflammation. Regeneration of the lymphatic capillaries is greatly retarded, however, by excessive fibrous tissue formation which slows down or completely hinders the growing vessels (Clark and Clark, 1932, 1937; Reichert, 1926; Eloesser, 1923). The regeneration of lymphatics in the healing of wounds will be further considered in Chapter 8.



(a)



(b)

FIG 9.—(a) Lymphatics of a normal mouse's ear, injected with hydrokollag (b) Greatly proliferated lymphatic capillaries in a mouse's ear shown up by hydrokollag injection 7 weeks after the end of 4 months' tar painting

(From Pullinger and Florey, 1937)

Contractility of Lymphatic Vessels

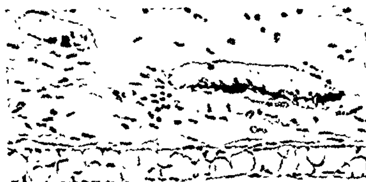
Everyone who has had experience in cannulating lymphatics will be familiar with the contraction of the walls of the large lymphatic ducts if they are not handled with care. Sometimes a large dilated duct will

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A



B



C



D

Fig. 10.—Histamine oedema in mouse's ear

A Widely dilated superficial lymphatics containing hydrokollag, stained iron haematoxylin and van Gieson

(From Pullinger and Florey, 1935)

contract to a mere thread and render cannulation impossible. Apart from response to mechanical stimulation, these large lymphatic ducts may undergo spontaneous contraction and relaxation. As long ago as 1869, Heller described rhythmical contractions of lymphatic vessels. These observations have been confirmed and extended by Florey (1927*a* and *b*) who showed that the lacteals (mesenteric lymph vessels) in the rat and guinea-pig were rhythmically contractile. These vessels contained smooth muscle fibres in abundance. On the other hand, no rhythmic contractions were observed in the lacteals of several other animals, viz. cat, dog, rabbit, squirrel, hedgehog, mouse, pig and man, although these vessels contracted when stimulated mechanically. They also contracted on stimulation of the sympathetic nerves or after the injection of adrenalin Florey (1927*c*) observed that the lymphatic trunks of the pleural surface of the diaphragm were rhythmically contractile in the guinea-pig but not in the rat and rabbit. Pullinger and Florey (1935) noted rhythmical contractions in the thoracic duct of the guinea-pig after a fat meal and of the lymphatic trunk draining the patellar tendon and testis in the rat and guinea-pig. Smith (1949) observed that in rats, mice and guinea-pigs the peripheral lymphatic vessels showed a spontaneous intermittent contractility which in rate was proportional to the rate of formation of lymph. Contractions could be initiated by an increase in the intraluminal pressure, and Smith postulated that the intermittent contractility was an important intrinsic mechanism contributing to the transport of lymph in these animals. In rabbits and dogs, however, this spontaneous contractility was not observed although, as is well known, the vessels contract when mechanically irritated In all these animals adrenalin and pituitrin produced an increased contractile rate or lymphatic spasm, while novocaine caused the cessation of movement together with dilatation.

In contradistinction to the larger lymph vessels the lymphatic capillaries are inactive. Pullinger and Florey (1935) injected with hydrokollag (a colloidal graphite suspension) the lymphatic capillaries on the surface of the testis and in the diaphragmatic pleura of the cat, and in the ear of the mouse. They found that these capillaries did not respond to mechanical stimuli, adrenalin or pituitrin. The lymphatics could, however, be passively distended by increasing the injection pressure, and on release of the pressure there was an immediate elastic recoil of the walls. While observing the small lymph vessels of the skin of the ear of the mouse outlined with India ink or hydrokollag over prolonged periods up to 5 hours, Hudack and McMaster (1932) never saw any contractions of the wall.

The cause of the dilatation of the lymphatic capillaries in oedema has given rise to much speculation. Whenever "free" fluid is present in

Lymphoid Tissue

In Lower Vertebrates

Lymphoid tissue occurs throughout the vertebrate series. In fishes, amphibia and reptiles it is closely associated with tissues giving rise to myelocytes and granulocytes, and, together with these cells, it constitutes the so-called lymphomyeloid organs.

Drzewina in 1905 made an extensive survey of lymphoid tissue in fish and in amphibia, and found that in these creatures it consists essentially of a cellular reticulum with both granular and non-granular leukocytes in its interstices. Lymphomyeloid masses may be found in one or more of a whole series of organs—kidney, liver, heart, genital glands, spleen, skull and alimentary canal. The association of lymphoid tissue with the alimentary canal is observed almost at the commencement of vertebrate evolution, and has persisted ever since. It is well marked in cyclostomes—e g. lamprey—in which the spleen has not yet evolved, being situated immediately under the mucous membrane, where it forms the so-called spiral valve, the main blood-forming organ in these animals It is an interesting speculation that the presence of substances stimulating erythrocyte formation—observed in recent years in the stomach (Sturgis and Isaacs, 1929), duodenum (Meulengracht, 1936) and small intestine (Uotila, 1938)—may perhaps be traced back to this primitive association of blood-forming tissue and intestine (cf. Hoff-Jorgensen and Landboe-Christensen, 1953; Landboe-Christensen, Berk and Castle, 1952).

In fish generally—apart from the cyclostomes—a new lymphoid organ appears, the spleen, and this organ persists throughout all the subsequent phases of vertebrate evolution, though its functions undergo some modification. The bone marrow first makes its appearance in amphibia. Conditions in reptiles are essentially the same as in amphibia, except that in the crocodile there may be a rudimentary accumulation of lymphoid tissue in the mesentery. It is not, however, until we reach birds and mammals that the lymphomyeloid tissues begin to show a really marked separation into their component elements.

In Birds

In gallinaceous birds and pigeons there are no lymph nodes In swimming birds they are present only in certain situations Fürther (1913), confirming the work of previous observers, found lymph nodes present in water, marsh and shore birds, but occurring without relation to any systematic classification. Two pairs of nodes were found, the larger pair—the cervicothoracic nodes—lying at the thoracic inlet. These were elongated structures surrounding the terminal part of the cervical

the interstitial tissues the lymphatic vessels are dilated, although the capillary blood pressure is greater than the tissue pressure, and the tissue pressure is in turn greater than the lymphatic pressure. The observations of Clark on growing lymphatic capillaries, and of McMaster (1947) and Pullinger and Florey (1935) on the lymphatics of the ear of the mouse suggest that as the connective tissue fills with oedema fluid and swells, the tension of the fibres attached to the lymphatic capillaries causes their walls to be drawn apart (Fig. 10). The alteration in calibre of the lymphatic capillary is, therefore, passive.

Phagocytic Properties of Lymphatic Vessels

Under normal circumstances the endothelial cells making up the walls of lymph capillaries in adult animals are not phagocytic. Clark and Clark (1938) in their observations on the lymphatic vessels in the transparent chambers in the ears of rabbits never saw any evidence of phagocytosis by the lymphatic endothelium. In contrast to the behaviour of the lymphatic endothelium in the adult, Wislocki (1916–1917) found that tadpoles kept in trypan blue for a week showed pronounced staining of the lymphatic endothelium. Clark and Clark (1918–1919) also showed phagocytosis of carbon and carmine particles by lymphatics in tadpoles; and later (1926–1927) described a growth process of lymphatic endothelium by which red cells were surrounded and eventually inside the capillary. MacCallum (1903) observed particles in the endothelial cells of the diaphragmatic lymphatics after introducing India ink into the peritoneal cavity, and Karsner and Swanbeck (1920–1921) made similar observations in the pleural lymphatics after the introduction of lampblack into the pleural cavity. These findings indicate that visible particles are phagocytosed occasionally by lymphatic endothelium in very young animals and under circumstances of chronic irritation, but there is no evidence of the constant participation of endothelial cells in the normal movement of material through the walls of the lymphatics.

Lymphatic Vessels and Lymph Nodes

Lymph nodes appear after the lymph vascular system has become fairly well organized, and their formation continues after birth, possibly even late in life. What is to become a node is at first a twisting meshwork of lymph capillaries. Blood vessels enter this net and later lymphocytes, the entire structure becoming a settling chamber and filter in the line of lymph flow, as well as a source of lymphocytes.

Lymphoid Tissue

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lymph duct. The second, or lumbar, pair was in the abdomen, just above the genital glands, on either side of the abdominal aorta.

As compared with the mammalian lymph node, the avian node is a *more primitive structure. Afferent lymph in birds drains into a central* channel, which is really a direct continuation of the afferent lymphatic vessel, and is surrounded by lymphoid tissue. Such channels have no reticulum. With the growth of the lymphoid tissue, the central spaces may become narrowed and irregular, germ centres may appear, though more often there are merely focal accumulations of lymphocytes. The capsule is only a thin layer of connective tissue and there is no trabecular formation; the cortical sinus is absent or rudimentary. The node is obviously grafted on to a lymphatic vessel and offers little obstruction to the flow of lymph.

However, the absence of lymph nodes in birds need not imply a deficiency of lymphoid tissue, which may in fact be found in situations where it does not normally occur in mammals. Thus Kondo (1937*a*, *b* and *c*) has shown accumulations of lymphoid tissue to be present in a number of birds, in association with the walls of lymph vessels. Biggs (1955) has confirmed that this holds good for the domestic fowl on a considerable scale, and has also shown that these accumulations of lymphoid tissue in the walls of lymph vessels may contain what appear to be germinal centres.

In Mammals

Classification. Wherever it is found in mammals, lymphoid tissue consists essentially of a mass of free cells—the vast majority of which are lymphocytes of various sizes—together with a supporting framework consisting of reticulum cells, and of fibrous, elastic and sometimes muscular elements. Ehrich (1929*a*), following Aschoff's classification, distinguishes three groups of lymphoid tissue:

1. Lymphatic tissue in the lymph nodes. This type of lymphatic tissue has afferent and efferent lymphatic vessels and is situated in the lymph stream
2. Lymphatic tissue in mucous membranes. Lymphatic tissue in the mucous membrane has only efferent lymph vessels and is situated in the fluid streams going from the mucous membrane into the interior of the organism.
3. Lymphatic tissue in the spleen. This type of lymphatic tissue has neither afferent nor efferent lymph vessels and is situated in the blood stream.

To these a fourth group may be added, the lymphoid tissue in bone marrow, considered in detail in Chapters 6 and 7. The problem of the

relationship between the thymus and the lymphoid tissues also calls for special consideration.

Separation from Myeloid Tissues and Association with Lymphatic Vessels

In mammals the segregation of the lymphoid from the myeloid tissues reaches its most advanced development with the widespread formation of the typical mammalian lymph node, intimately associated with lymphatic vessels. In lower vertebrates there are present lymphatic vessels, but not lymph nodes. The two become connected later on in vertebrate evolution. This must mean that the primary function of the lymphoid tissues, whatever it may have been, was originally independent of the lymph stream.

It must be clearly understood, however, that though the separation of lymphoid from myeloid tissue is more marked in mammals than in other vertebrates, it is still not complete. Lymphoid nodules occasionally, and scattered lymphocytes always, are to be found in mammalian bone marrow. At times also myeloid cells may be found in lymphoid tissue, constituting the so-called myeloid metaplasia (see Lang, 1938). The problem of the lymphocyte in bone marrow is discussed in detail in Chapter 7. As to myeloid metaplasia, our chief interest in the condition is for the possible light which it may throw on the ability of lymphocytes to become transformed into other cells. A review of the literature shows that the subject is still the battleground of conflicting haematological theories. The mere fact, however, that lymphoid and myeloid cells can flourish side by side is significant, since it perhaps disproves the view that there is an essential biological antagonism between the two cell groups.

The Lymphoid Organ

When we consider the total amount of lymphoid tissue present in the organism—the lymph nodes of the limbs, the intestinal and mesenteric lymphoid tissue, the spleen, the thymus, the nasopharyngeal aggregates, and the cervical and thoracic nodes—it is clear that if all this lymphoid tissue could be collected into a single compact mass it would form an organ of no inconsiderable size. Bone marrow, which is also characterized by the diffuseness of its distribution, presents a situation similar to that of lymphoid tissue. Estimations of the total volume of bone marrow have been made for the most part by a method depending primarily upon the resistant nature of the bones within which the marrow is contained, and after obtaining an estimate of total marrow volume, further corrections may then be applied to give the figure for red bone marrow, which has been variously estimated to be 2·6 per cent of the body weight (Töppich, 1914, human infants); 1 7–2 9 per cent (Mechanik,

1926, human adult); 1·7 per cent (Nye, 1931–1932, rabbit); 3·02 per cent (Fairman and Corner, 1934, total marrow, albino rat); and 2·00 per cent (Hudson and Yoffey, 1954, guinea-pig). Dietz (1944) made use of a somewhat novel method for the determination of marrow volume, and his results are of the same order as those obtained by other workers.

The only method, however, available to determine the total amount of lymphoid tissue has been the careful dissection and weighing of all the scattered lymphoid masses. Hellman (1914) and Sjovall (1936) have done this for rabbits, and Andreasen (1943) for rats. Hellman's figures for lymphoid tissue average approximately 0·5 per cent of the weights of his animals, while Sjovall's figure is 0·14 per cent. Both are underestimates. They do not include the thymus, since its position as a lymphoid organ was regarded as open to doubt. But even apart from the thymus, there are numerous small and scattered outlying nodules of lymphoid tissue which gross dissection methods would fail to detect. An excellent example of this is afforded by the lymphoid follicles in the human large intestine. Dukes and Bussey (1926) found in the large intestine many tiny lymph follicles, 1–2 mm. in diameter. On the average there were 3·5 follicles per sq. cm. of mucous membrane, with a count for the entire large intestine of 2,351 follicles in one subject, 4,618 in another. These results were only obtained by a special technique of staining and clearing the intestine *in toto*. If all such scattered lymphoid masses could be taken into account, together with the lymphocytes dispersed throughout the connective tissues and bone marrow (see Chapter 7) it is quite possible that the total lymphoid tissues would be of the order of 1 per cent of the body weight.

Andreasen (1943) gives extensive data for rats of various ages. In the young adult rat the total lymphoid tissue, including the thymus, he estimates to be somewhere between 0·5–1·0 per cent of the body-weight. Andreasen emphasizes, however, that the weight of the lymphoid organ "affords only an inadequate measure for its content of the specific tissue" (i.e. lymphocytes), and he makes use of the nucleic acid phosphorus content as a more reliable guide to the lymphocyte concentration. The point is worth emphasizing, since throughout the literature one repeatedly *finds the weight* of lymphoid tissue being *regarded* as directly *proportional* to its lymphocyte content, whereas it is in fact by no means certain that such *is invariably the case.*

The view that the various scattered lymphoid masses may be regarded as a single functioning organ is based on the assumption that there are [illegible] in the [illegible] owever [illegible] ipheral lymph nodes of rabbits contained monocytes, whereas the mesenteric

nodes did not. Differences between mesenteric and other nodes have also been noted in connexion with fat absorption (see Chapter 6); and Job (1922–1923) has expressed the opinion that the lymph sinuses are better developed in abdominal and thoracic nodes than in nodes elsewhere. Kindred (1938) in the rat found that plasma cells were much more numerous in the medullary cords of the submaxillary nodes than elsewhere, while the inguinal nodes were distinguished by the occurrence of nests of differentiating eosinophilic granulocytes. It may well be [illegible] main the waste products of tissue activity. The mesenteric nodes occupy, with respect to the lymph stream, much the same position as the liver does with respect to the blood. In either case the structure has, as it were, first choice of the products of digestion (see Dabelow, 1930–1931; Hansen, 1944), and the suggestion has been made by Sanders and Florey (1940) and others that the mesenteric nodes are the main source of the thoracic duct lymphocytes entering the blood (See Chapter 6. *Vide* also Nordmann, 1928).

Changes due to age. Lymphoid tissue is present in mammals throughout life, but its amount seems to undergo changes with age. According to Harris and his associates (1930) lymphoid tissue in man reaches its maximum development about the twelfth year, and then undergoes shrinkage—at first marked and then more gradual—until finally a more or less stable condition is reached. "More or less" may be a somewhat inadequate description, but we do not possess sufficiently accurate quanti- [illegible] the Malpighian follicles in general behaves more or less like the lymphatic tissue elsewhere in the body at different ages". (See also reviews by Krumbhaar, 1939; Denz, 1947; and Andrew, 1952.)

Sex differences. The endocrine control of the lymphoid tissues will be discussed more fully in Chapter 6, but it should here be noted that differences between the lymphoid tissues in male and female have been described. Thus according to Andreasen, Engberg and Ottesen (1945), and Andreasen (1946), the total amount of lymph node and splenic tissue in the guinea-pig is greater in the female than in the male. On the other hand Reinhardt (1946), working with rats, found a tendency for the lymphoid tissue to be heavier in the male than in the female. The effect of castration upon the thymus and lymph nodes is to cause considerable post-puberal enlargement in the male (cf. Chiodi, 1938; Ross and

Korenchevsky, 1941, Baker, Ingle and Li, 1951) but not in the female

Thymus

The position of the thymus in relation to the lymphoid tissues has long been a matter of controversy. Certain points of difference are immediately apparent. Thus the thymus contains epithelial, non-lymphoid elements. On the other hand it does not normally contain plasma cells and hence it is not surprising that extracts of thymus are devoid of antibody (Bjørneboe, Gormsen and Lundquist, 1947; Harris, Rhoads and Stokes, 1948). Difficulty arises in connexion with the small thymocytes, which are present in the thymus in such large numbers, and which, though considered by many to be of epithelial origin, are morphologically indistinguishable from the small lymphocytes (Hammar, 1905, 1921 and 1936; Pappenheimer, 1913; Whitney, 1928; Bargmann, 1943; Baillif, 1949). It is true that slight differences have been noted, but they are not decisive. Thus Downey (1948) pointed out that "the smallest lymphocytes with very dark nuclei with coarse chromatin blocks and little or no cytoplasm are more numerous in thymus than in node". Dustin and Gregoire (1932) and Gregoire (1932*a*) have attributed the small size of the thymocyte to repeated rapid mitoses. Smith and Thomas (1950) observed that in new-born mice about 40 per cent of the thymocytes contain glycogen granules, but the number of these cells drops abruptly between 14–18 days, and by the 29th day only an occasional glycogen-containing cell can be seen. It would appear that the presence of glycogen granules is merely a transient phenomenon in an otherwise unchanged cell type.

According to Kindred (1938) the small lymphocytes in the thymus have a low but definite mitotic rate, whereas in other lymphoid tissues mitosis of small lymphocytes occurs rarely if at all. It is to be noted that in tissue cultures thymocytes show the same distinctive type of movement as do lymphocytes (Murray, 1947). Furthermore, as judged by the response to thymo-toxic serum, the thymocyte and the small lymphocyte are also identical (Pappenheimer, 1917).

Differential response of thymus and other lymphoid tissues

However, an element of uncertainty has arisen about thymocytes and lymphocytes being identical since the thymus often appears to react differently from lymphoid tissues elsewhere. References to earlier work on the differential response of thymus and other lymphoid tissue will be found in the papers of Margolis (1930), Wiseman (1931*a*) and von Albertini, Gasser and Wuhrmann (1935–1936). Thus during inanition both

thymus and other lymphoid tissues undergo considerable diminution, the former more markedly and rapidly than the latter (Jolly, 1914, and many others; for bibliography see Andreasen, 1943). On the resumption of feeding all these tissues return to normal, but the thymus lags quite appreciably behind the others. Warren, McMillan and Dixon (1950) reported that in mice which had been given small doses of P^{32} there was a secondary hyperplasia of thymus, spleen and bone marrow but not of lymph nodes. Tullis (1951) noted that after irradiation there was less damage to lymphocytes in thymus than in lymph nodes.

The extensive work of Selye and his collaborators on the response of the thymo-lymphatic system to stress (summarized by Selye, 1950) indicates that the earliest and most marked changes occur in the thymus. Baker, Ingle and Li (1951) showed that in male rats, which were given ACTH 8 times per day for periods up to 21 days, changes appeared in the thymolymphatic system similar to those elicited by stress, but "whereas thymocytes were removed almost completely from the thymus by a 1 mg. daily dose, 3 or 6 mg. administered by continuous injection was required to deplete the splenic corpuscles of their lymphocytes. . . . In the lymph nodes of rats similarly treated, cortical nodules were still present and contained lymphocytes of practically normal nuclear structure." The great sensitivity of the thymus to ACTH has been used for the assay of that substance (Bruce, Parkes and Perry, 1952).

However, in the case of stress or the administration of ACTH the difference is one of degree and not of kind. That is to say, the thymus responds in the same manner as the other lymphoid tissues, but differs from them in responding more rapidly and completely to smaller doses of ACTH or to lesser degrees of stress. This is in marked contrast with a number of instances in which the thymic response appears to be diametrically opposed to that of the lymphoid tissue. Thus Money, Fager, Lucas and Rawson (1952) reported that in rats compound L depressed the weight of the thymus, but increased that of the mesenteric lymph node. Similarly Money, Fager and Rawson (1952) found that testosterone also diminished the weight of the thymus but increased that of the lymph node. Marder (1949) noted that in intact mice treated with thyroxine the weight of the lymph node increased while that of the thymus decreased, whereas in adrenalectomized mice similarly treated both lymph nodes and thymus gained in weight. Savard and Homburger (1949) found that after transplanting sarcoma 180 into mice the thymus underwent atrophy, the lymph nodes hypertrophy. Shrewsbury and Reinhardt (1955) observed that castration in male rats causes increase in weight of the thymus but not of the cervical or mesenteric lymph nodes, whereas if the adrenals are first removed there is a marked weight increase in both. In view of what we have previously said about the significance

of weight changes in lymphoid tissue, it is clear that all these experiments must be interpreted with a certain amount of reserve.

Osogoe and Hitachi (1950) intravenously injected into rabbits saline suspensions of thymocytes from other rabbits, and found that they accumulated in the liver and spleen of the recipient in the same way as did suspensions of lymphocytes derived from lymph nodes. Further discussion of these lymphocyte transfusion experiments will be found in Chapter 6. From the fact that thymocytes accumulated in the interlobular connective tissue of the liver and in the perifollicular regions of the spleen, in the same way as lymphocytes, they inferred that the biological properties of thymocyte and lymphocyte were identical.

Status Lymphaticus

The occurrence of status thymicolymphaticus (Paltauf, 1889), with simultaneous hypertrophy of lymphoid tissue and of thymus, was formerly regarded as evidence of identity between lymphocyte and thymocyte. So much doubt, however, has been cast on the existence of this condition as a clinical or morphological entity (see for example Hellman, 1943) that it seems unwise to base any such conclusions upon it. It must however be regarded as significant that subtotal thymectomy in newborn guinea-pigs is followed by a generalized hyperplasia of lymph nodes all over the body, and also of Peyer's patches—though not of the spleen. Although this hypertrophy is transitory, it is quite definite (Gyllensten, 1953), and it seems difficult to explain on any other basis than that one is dealing with the compensatory hypertrophy of a similar tissue Gyllensten and Ringertz (1954), using the P^{32} uptake as a more sensitive indicator of lymphoid tissue activity, found that they could detect increased lymph node activity within four hours after subtotal thymectomy, a finding which they believe " partly supports the view that the lymph node hyperplasia was due to an increased lymphocyte production inside the lymph node tissue investigated ".

Lymph Nodes

General arrangement. Although all mammals possess lymph nodes, the number and the grouping of the nodes show marked species variations. In any given species, however, the nodes appear to be more constant than is generally realized. As compared with other mammals, the lymph nodes in the monkey and in man seem to be smaller but more numerous than in lower animals. In animals such as the dog, cat and rabbit, for example, the mesenteric lymphoid tissue tends to form one large node, the pancreas of Aselli. In the rabbit this node weighs three to four times as much as all the other lymph nodes in the body (Hellman, 1914). In the rhesus monkey and in man, on the other hand, there is

no pancreas of Aselli, but instead a large number of small mesenteric nodes. One gets the impression of a definite evolutionary trend towards the splitting up of a given amount of lymphoid tissue into many small nodes instead of the continuing occurrence, as in the lower forms, of a few large ones.

The principle involved is best illustrated if one reviews the deep cervical lymphatic pathway in the cat, dog, rabbit, monkey and man. The pathway is well defined, commencing in the mucous membrane of the nose and pharynx. Its numerous tributary lymphatics join to form a single duct, which runs down the side of the neck on the outer side of the carotid artery. At varying levels in the neck—in the dog, just below the angle of the jaw, in the cat and monkey lower down—the deep cervical lymph duct receives the superficial lymphatics. As far as the deep pathway is concerned, there is always at least one lymph node along its course, and through this node all the nasopharyngeal lymph passes. In numerous dissections we have never seen a lymphatic vessel by-passing the node without traversing it (Yoffey and Drinker, 1938). This is in agreement with the findings of Drinker, Field and Ward (1934) for lymph from the leg—namely, that lymph never reaches the blood without passing at least one node.

In the dog, cat and rabbit, there is usually only a single node along the course of the deep cervical duct, whereas in the monkey there are five or six, and in man even more. The pathway is completely interrupted by each of these nodes. The pathway of lymph through a chain

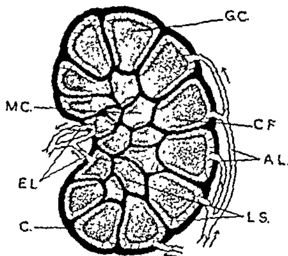


FIG 11—Diagram illustrating the architecture of a lymph node

A.L., afferent lymphatics, *E.L.*, efferent lymphatics, *C*, capsule, *L.S.*, lymph sinus, *C.F.*, cortical follicle, *G.C.*, germinal centre, *M.C.*, medullary cord

of nodes, instead of a single node, must undoubtedly make for greater filtering efficiency, though we are unable to say that this is the sole or the main purpose of such passage. But whatever the reason for the difference, it is very striking from a morphological point of view to find a chain of many small nodes taking the place of a single large one.



FIG 12—1 and 2, low-power photomicrographs of injected popliteal lymph nodes (× 6), 3, the distribution of the injection mass in the cortical and in the beginnings of the intermediary sinuses (× 26), 4, intermediary sinuses in the medulla of an injected node (× 128).

(From Drinker Wislocki and Field, 1933)

Structure. Figure 11 represents diagrammatically the general plan of lymph node structure, and the relation to the node of its afferent and efferent lymphatic vessels. The course of lymph flow through a node is well brought out by perfusion with India ink (Fig. 12) Ink flows first into the cortical sinus, and from there through the medullary sinuses into the hilum and afferent vessels. Both cortical and medullary sinuses are traversed by a reticulum, consisting of cells which not only hold up the carbon particles mechanically but also are actively phagocytic. The cells lining the sinus walls, though resembling flattened endothelial cells elsewhere, are also phagocytic (Fig. 13 and 14). In the medullary sinuses the walls are thin but complete and ink flowing through the sinuses runs between quite definite borders, beyond which it does not escape. In regions of active lymphocytic growth the walls of the sinuses become incomplete, and ink in the sinuses then passes for varying distances into the adjacent lymphocytic collections. This state of affairs is found most frequently in the cortical sinuses.

But though Fig 11 may be taken as a useful guide to the structure of a lymph node, and suffices for a consideration of certain aspects of nodal function, it is to be regarded as only a first approximation. Fuller information about the great range of variation in lymph node structure may be obtained from the review by Hellman (1943). In many species, including man, neither the lymph sinuses nor the trabeculae are as conspicuous as in the dog, the animal on which schematic diagrams such as Fig. 11 are usually based. Furthermore it has been claimed (Job, 1922–1923; Furuta, 1947) that not infrequently an afferent lymph vessel may by-pass a node, and continue into an efferent channel without traversing a sinus. Though we ourselves, as already noted, have never actually seen this happen—either in a uninodal or a multinodal pathway—it is obviously difficult to establish a universal negative. It can, however, be stated with a fair degree of confidence that the by-passing of a node is a most infrequent occurrence.

As far as human nodes are concerned, Denz (1947), after examining over 300 lymph nodes from autopsies, as well as a small number of biopsy specimens, comments that the continuity of the marginal sinus is interrupted at various points by the lymphoid tissue coming close to the capsule. Furthermore, he confirmed the view of Heudorfer (1921) that the cortical lymphoid tissue is a continuous mass which is only pierced by the lymph sinuses, but not interrupted and divided into segments as Fig. 11 would suggest. Denz also emphasizes, as indeed do many other workers, that there is no sharp boundary between cortex and medulla, and that the distinction between these two zones becomes especially difficult in the more deeply situated nodes, where the cortex reaches irregularly into the interior of the gland.



FIG 13 —*Top*—Camera lucida drawing of an intermediate sinus injected with a very dilute India ink solution Note the phagocytosis by the reticulo-endothelial cells in the sinus and in the wall, and also the flattened cells which line the sinus with fair completeness (× 390), *Bottom*—Camera lucida drawing of the rectangle marked in the upper figure, showing details of reticulo-endothelial cells lining the sinus (× 1000)

(From Drinker, Wislocki and Field, 1933)

The cell population of lymph nodes. The most numerous of the cells found in lymph nodes—and in most other lymphatic tissues—are lymphocytes. The small lymphocytes, with little cytoplasm and pachychromatic nuclei, stain darkly with the customary stains, while the medium and larger lymphocytes stain less deeply (Fig. 15). Besides the lympho-

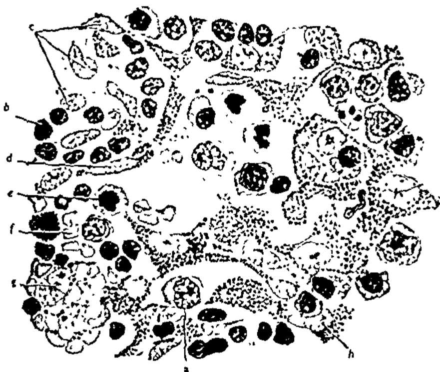


Fig [illegible] lymph node of a rabbit which had received [illegible] carmine Note the non-phagocytic [illegible] stain × 950 *a*, large lympho- [illegible] *d*, macrophage lining sinus ; [illegible] crophage containing erythro- [illegible]

(From Maximow and [illegible] Histology, 6th Edition, 1952, W B Saunders Co, Philadelphia)

cytes, the main cell groups to be found are the phagocytic cells, and the primitive reticular cells. Plasma cells may on occasion be quite numerous, especially in the medullary cord, while granulocytes are normally present in only small numbers.

Phagocytic cells are found both in the sinuses, where they are either fixed or free, and also scattered throughout the lymphoid tissue. In the sinuses they occur (1) as a network of cells, with branching processes, the

9



FIG [illegible]

(× 1000)

(From Drinker, Wislocki and Field, 1933)

ring in lymphoid tissue may be small, medium or large (Fig. 14). There are no very definite measurements for these groups (p. 409); all three cell types are also met with in peripheral blood, though the large lymphocyte may be sometimes difficult to find. Lymphocytes in lymphatic



Fig. 16.—Heteroplastic development of lymphocytes from primitive reticular cells in a human lymph node. Haematoxylin eosin-azure II. × 750. *a*, small lymphocytes; *b*, medium lymphocytes; *c*, large lymphocyte; *d*, primitive reticular cells; *e*, mitosis in medium-sized lymphocyte; *f*, dividing primitive reticular cells.

(From Maximow and Bloom: *A Textbook of Histology*, 6th edition, 1952. W. B. Saunders Co., Philadelphia.)

tissue may occur either evenly diffused, or else in characteristic accumulations, the lymphoid nodules (Ehrich, 1929; Gyllensten, 1950). These nodules may be present in all the lymphoid tissues, though their occurrence in the thymus is rare. In the intestine they occur under the mucous membrane, more especially of the distal portion of the small intestine, either as solitary nodules, or as aggregated nodules (Peyer's patches). They also occur in lymph nodes and in the spleen. A simple type of nodule, which may perhaps be termed the primary nodule (the "solid"

sinus *reticulum* (2) as the endothelium lining the walls of the sinuses, where they are known as *littoral* cells, or (3) as rounded macrophages floating freely *in the lymph (Figs. 13 and 14). In the lymphoid masses* they stand out readily as large lightly staining cells among the smaller, more deeply staining and closely packed lymphocytes, especially in the germinal centres where they give rise to the appearance sometimes described as "pitting" These cells frequently contain small darkly staining masses in their cytoplasm which appear to be the nuclei of ingested dead cells, probably lymphocytes, for the most part, and are referred to by Flemming (1885) as "tingible Korper".

The *reticular* cells are found scattered irregularly among the lymphocytes in diffuse lymphoid tissue. In the germinal centres, they tend to be more numerous and may either form a true reticulum or become detached and free (Fig. 16) Their nuclei are poor in chromatin; their cytoplasm is not abundant, staining but *moderately as a rule with cyto*plasmic stains and containing few or no inclusions. These cells are not phagocytic but may readily become so.

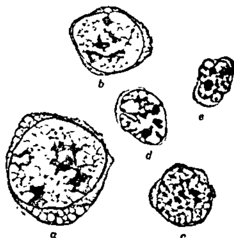


FIG 15 —Lymphocytes from a section of a human lymph node As compared with smear preparations, much of the fine nuclear detail is lost

a large, *b*, *c*, medium-sized and *d*, *e*, small lymphocytes Haematoxylin eosin-azure stain × 1500

(From Maximow and Bloom, *A Textbook of Histology*, 6th edition 1952, W B Saunders Co, Philadelphia)

The reticular cells are now generally regarded as primitive undifferentiated cells, capable of transforming into other cells, such as macrophages, plasma cells, or lymphocytes, though under normal circumstances they give rise mainly to lymphocytes The heteroplastic formation of lymphocytes from reticular cells was first established by Downey and Weidenreich (1912), and subsequently in a number of publications by Downey and his pupils (see for example Sundberg and Downey, 1942) Considerable numbers of reticular cells may be seen at times in the germinal centres, as in Figs 15, 16, though they also occur in diffuse lymphoid tissue. Being primitive and undifferentiated, the reticular cells are believed to be multipotential and capable of transforming not only into the cells already mentioned, but also less frequently into cells of the myeloid or erythroid series.

Lymphoid nodules. We have already noted that the *lymphocytes* occur-

even be the case that the absence of such stimuli was responsible for the poor condition of the animals in the experiments of Glimstedt discussed later. The early experiments of Ehrich (1929*b*) seem to fit in with such an interpretation. Ehrich gave rabbits intravenous injections of killed staphylococci, and observed that "the highest development first attained of Flemming's secondary nodules in the spleen coincided with the highest point of the lymphocytosis". But since the secondary nodules then continued to develop though the lymphocytosis subsided, he concluded that the nodules could not be responsible for lymphocyte formation. However, the rate of production of lymphocytes is not the only factor determining their level in the blood, and the later studies of Ehrich and his group (Ehrich and Harris, 1942; Harris, *et al.*, 1945; Ehrich, Drabkin and Forman, 1949) forcibly bring out this point through observations on the lymphocyte content of lymph draining enlarged nodes, with hypertrophy of the germinal centres.

Ringertz and Adamson (1950) made a detailed study of the histological changes in regional and non-regional nodes, as also of the spleen, following the subcutaneous injection in guinea-pigs of bacterial antigens. Their experiments showed a biphasic response of the lymphoid tissue. In the first phase, beginning on the first or second day, they found a diffuse hyperplasia of cortical lymphoid tissue. At times ranging from the 10th to 16th day the second phase supervened, marked by the new formation of many large germinal centres, which remained active until the end of the injection period and only began to regress a week after the cessation of the injections. They noted that broth alone could evoke the response, thus recalling the earlier experiments of Grégoire (1932*b*) with horse serum, which suggested that the lymph node reaction might primarily be due to the presence of a foreign protein. They concluded by agreeing with Conway (1937) about the occurrence of cyclic changes in the lymphatic nodules (Fig. 17), and by accepting the essentially germinal and proliferative nature of the centres.

The work of Ehrich and his collaborators has served to emphasize one very fundamental point, namely that whether multiplication of lymphocytes occurs mainly in the germinal centres, or in the diffuse lymphoid tissues, the lymph nodes as a whole are active lymphocytopoietic organs. This aspect of lymph node function is further discussed in Chapter 6

The appearance in the germinal centres of dead lymphocytes, ingested by macrophages, might seem at first to be strongly in favour of the reaction hypothesis But as De Bruyn (1948) comments, these degenerative processes are to be seen in germinal centres which show signs of great mitotic activity. Furthermore, the association of cell death with proliferative processes is by no means limited to the germinal centres of

nodule of Ehrich, 1929) consists of a focal accumulation of small or medium lymphocytes. This type of nodule occurs frequently in new-born guinea-pigs (Gyllensten, 1950). Nodules with more than one cellular zone are best described as "secondary" nodules. For a full discussion of the confused terminology of the various lymphoid nodules, reference should be made to Ehrich (1929*a*) and Gyllensten (1950).

Germinal centres and reaction centres. Cyclic changes. Of the various lymphoid nodules, the one which is most frequently found and which is most characteristic consists of a clear central portion surrounded by a dense rim of small lymphocytes. The clear central portion was described by Flemming in 1885, and was called by him the germinal centre because of the numerous mitoses which it contained. The daughter cells after division would be pushed out to form the peripheral layer of small lymphocytes, which subsequently entered the lymph sinuses, and once in the lymph stream would be carried in it to the blood. It has been a source of confusion that the term "secondary" nodule has been applied both to the clear germinal centre and to the centre with its surrounding layer of small lymphocytes Ehrich (1929*a*) also described a "pseudo-secondary nodule", consisting of a central collection of small lymphocytes, surrounded by a paler zone of large cells.

Flemming's view of the significance of the germinal centre raises a number of problems. Germinal centres are not essential for lymphocyte formation, since active multiplication of these cells occurs before birth at a time when centres are not present. Germinal centres appear in early post-natal life (Gyllensten, 1950, 1954) and even when they have appeared they are subject to cyclic changes (Conway, 1937). Furthermore, even in the adult mammal active multiplication of lymphocytes may still take place in diffuse lymphoid tissue completely devoid of germinal centres. For these and other reasons, Flemming's original interpretation of the secondary nodules as germinal centres has been disputed by many workers, notably Hellman (1939, 1943), who maintains that they are centres of reaction to noxious substances

The position becomes still further complicated by the fact that, as noted by Rohlich (1928) in the cat, Taliaferro and Cannon (1936) in the monkey, and Kindred (1938) in the rat, the germinal centre frequently consists of two distinct zones, namely an inner active portion, with many medium lymphocytes, and an outer inactive portion, near the capsule. It is the outer portion which is usually capped by a closely packed zone of small lymphocytes

It is difficult to assess the precise significance of bacteria or bacterial toxins in relation to lymphoid tissue (see Chapter 5), and Gyllensten (1954) suggests that within limits bacteria and their products may actually function as normal morphogenetic stimuli (cf. Grégoire, 1945). It might

While there is no doubt that the germinal centres are readily damaged so as to show abnormal degenerative changes, it seems to us that on the whole the normal germinal centre is best regarded in terms of Flemming's original conception, with the modifications suggested by Conway (1937), Rohlich (1928) and Kindred (1938). As Conway observes, apart from instances of overwhelming damage to the germinal centre, the appearance so typical of the reaction centre is only seen after a period of intense lymphocytopoietic activity. If this view is correct, the centre thus named is at times not so much one of reaction as of exhaustion, albeit only temporary, since another focus of lymphocytic proliferation may subsequently develop within it.

In the case of the thymus, the problem of the germinal centre does not arise, though that of the heteroplastic formation of lymphocytes calls for consideration. The problem has recently been reviewed by Bargmann (1943) and Bailiff (1949) in an experimental study of degenerative and regenerative changes in the thymus of the rat following the injection of acid colloidal dyes. The part played by the endothelium of the blood capillaries is not at all clear. In the opinion of many workers (e.g. Maximow, 1925*b*) the vascular endothelium is relatively inert, capable of giving rise only to fibroblasts. On the other hand a number of investigators (e.g. Gillman and Gillman, 1949) have concluded that the endothelium of the blood vessels, more especially the venules, "can hypertrophy, round off, and become discrete entities". These mobilized endothelial cells are regarded by the authors just quoted as the main stem cells for the lymphocytes in the normal node, though under pathological conditions they may become transformed into other cells. Furthermore, stem cells are thought to be capable of arising from capsular fibroblasts, and also from "lymphocytes, fibrocytes, histiocytes and polyblasts" (Gillman and Gillman, *loc. cit*)

Cellular equilibrium in lymph nodes. In the normal lymph node—an entity which is somewhat difficult to define, but which we all think we can recognize—there is some sort of equilibrium between the different cell groups. This equilibrium alters with age, diet and hormonal influences, though the precise nature of the changes may not be altogether clear. Bailiff (1951) examined the effect on rats of repeated injections of Biebrich's scarlet, chlorazol black E and colloidal mercuric sulphide. These substances appeared to stimulate the development of phagocytic cells in the lymph nodes, after a period of several days—the "activation period"—during which the lymphocytes diminished through depression of mitosis, accompanied by the usual removal of lymphocytes via the lymph stream. Lymphocytopoiesis did not cease completely, but continued at a lower rate, and if the substances were administered in subtoxic doses, "it is possible to secure some degree of equilibrium in the

lymphoid tissue, but occurs in a number of other situations. It is of interest that Ringertz and Adamson (1950) also, in the experiments just quoted, found that signs of cell death were conspicuous in germinal centres at the peak of their lymphocytopoietic activity.









FIG 17.—Photomicrograph showing four nodules at different stages of development [illegible]

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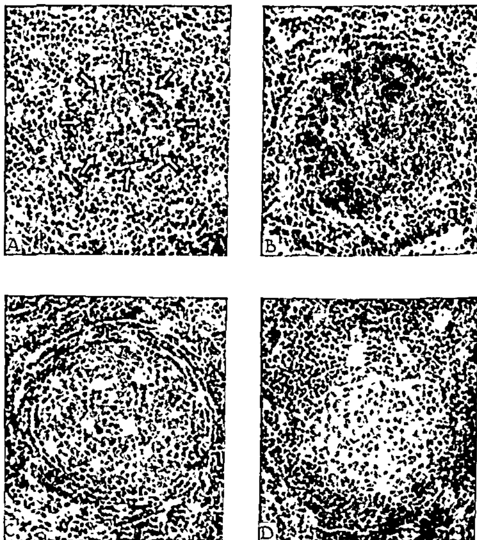


Fig 17—Photomicrograph showing four nodules at different stages of development

occur in response to altered nutrition and to other stimuli, such as the parenteral administration of foreign protein (see Chapter 6). In such cases we are dealing with increase in size of lymphoid tissue already present. The question arises whether, in the adult, there can be a completely new formation of lymphoid tissue, more particularly of lymph nodes. The problem has been investigated in conditions of pathological irritation, and in the normal animal following the experimental extirpation of lymph nodes.

Observation of lymph nodes in pathological conditions has suggested very strongly that new formation of nodes may occur. Bayer (1885) pointed out that, in carcinoma of the breast with axillary metastases the number of diseased nodes in the axilla appeared to be far greater than the number of healthy nodes present in the normal axilla; while in addition, surrounding the diseased nodes, there were many normal ones, small and apparently newly formed. Bayer performed extirpation experiments in four dogs from which he removed the axillary nodes. At varying intervals afterwards he examined the area of operation, and found accumulation of cells, which he interpreted as developing lymph nodes. These cell masses, however, did not possess a typical lymph node structure, nor can one be certain from Bayer's description and illustrations that they were essentially lymphocytic in nature. Bayer was of the opinion that these so-called nodes were developed largely through the transformation of fat cells.

We know that nodular accumulations of lymphocytes can appear in the connective tissue in various conditions, but lymphocyte masses are not lymph nodes. New formation of lymph nodes could occur if such masses secondarily acquired a connexion with neighbouring lymphatic vessels. Conversely, new nodes could conceivably develop if lymphoid tissue were formed in the neighbourhood of lymphatic networks.

Following Bayer's first observations numerous workers attempted to solve the problem by experimental means. Though nodes in various parts of the body have been chosen for investigation, most workers appear to have selected the popliteal node for extirpation experiments. This is because there is usually only a single node, and the possibility is thus excluded that enlargement of another node or nodes, not noticed at the time of operation, might be mistaken for regeneration of the one removed. The earliest experiments (e.g. Bacialli, 1886; Ribbert, 1889) dealt with the results of partial extirpation of nodes. There was agreement that regeneration could occur from the part left behind, but disagreement concerning the actual source of the re-formed tissue. Fat, lymphatic endothelium, fixed tissue cells, were suggested as possible elements from which new lymphoid tissue developed. It would perhaps appear simplest to explain the results of partial node resection as due to hypertrophy of the

node between phagocytosis and phagocytogenesis on the one hand, and lymphocytogenesis on the other". However, this equilibrium cannot be maintained indefinitely, and after a while lymphocytogenesis is restored to its normal level of activity, while macrophage formation diminishes. During this period "the depressing effects of the colloid are no longer effective in suppressing lymphocyte mitoses". These experiments then reveal a disturbance of the normal cell balance by a shift, albeit temporary, towards macrophage production.

Gillman and Gillman (1949), *after giving rats 16 fortnightly injections* of 1 ml of 1 per cent trypan blue, found the lymph nodes enlarged to the point where they almost become converted into tumours, in which they found that "the general organization and the cell population of the normal node can be radically changed. The lymphocytes are replaced in part or completely by polyblasts, plasma cells, histiocytes, paramonocytes, mobilized endothelial cells, or by a variable combination of these different cells". When one compares these experiments with those of Baillif (1951), the "activation period" for some reason had evidently continued without any intermission. Furthermore it is abundantly clear, from experiments of this nature, that we are as yet ignorant of the basic mechanism for controlling the cellular equilibrium of the normal lymph node.

Contractility Numerous observers have described the presence of smooth muscle, in varying amounts, in the capsule and trabeculae of lymph nodes (His, 1862; Hellman, 1943); and the question arises whether contraction of this muscle plays a significant part in the propulsion of lymph, or expelling lymphocytes from the node. We have repeatedly perfused the popliteal lymph node of a dog with physiological salt solution, to which after a while adrenalin was added, but have never observed evidence of contraction

This finding conflicts with the observations of Florey (1927*a*) and of Martin (1932) on cat and dog isolated mesenteric nodes suspended in Ringer solution Florey and Martin both noted that the addition of adrenalin usually resulted in contraction The possibility that this contraction might have been due to the contained blood vessels was considered by Florey, who found that isolated pancreas and other organs which also contained blood vessels did not contract after adding adrenalin. Contraction of smooth muscle in lymph nodes would no doubt assist both the flow of lymph and the movement of lymphocytes (cf. Florey, 1927*a*), but it does not appear to be essential for either of these processes. Comparison with the spleen at once suggests itself; but there is no real analogy, for though the spleen contains lymphoid tissue, its well developed musculature seems to be associated with the rapid changes in size necessary for the discharge of its reservoir function.

Regenerative powers. Variation in the amount of lymphoid tissue can

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residual nodal tissue, which was indeed the explanation put forward by Rouviere and Valette (1933), who attributed the hypertrophy to a regulatory influence of the lymph flow.

With regard to the results of total extirpation, there was a lesser measure of agreement. Some observers (e.g. Meyer, 1906; Vecchi, 1911; Rouviere and Valette, 1937) maintained that no regeneration occurred after complete removal of nodes, while others (e.g. De Groot, 1912; Auché, 1930) concluded that regeneration was the rule rather than the exception.

In recent years, Furuta (1947) has re-investigated the problem. Like many previous workers, and for the same reasons, he selected the popliteal node This was removed surgically in 152 rabbits, ranging in age from 3 days to 3½ years. In many cases it was removed bilaterally, so that the observations were finally based on a total of 270 specimens.

Furuta found that in adult animals, from 8–15 months old, regeneration of an excised node practically never occurred It did however occur in about 50 per cent of young and nearly adult animals The best results, both in regard to frequency of regeneration and to the degree of structural differentiation in the newly formed node, were obtained in animals from 45 to 60 days old. The first change was the re-establishment of continuity in the lymphatic vessels, in the vicinity of which there subsequently appeared small islands of mesenchymal cells, which Furuta called lymphoreticular tissue From this tissue mass there then grew a lymphatic nodule, which after a further 3–4 weeks became converted into a true node. Furuta however emphasized that in one fundamental respect the regenerated nodes departed from the normal. They possess "a lymphatic bypass or shunt", a feature which was found to be specific for them.

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CHAPTER 2

THE FORMATION OF LYMPH

In the last century, when it was thought that the lymphatic vessels communicated directly with the tissue spaces, the terms "tissue fluid" and "lymph" were used synonymously. It is now generally accepted, however, that by "lymph" we mean only that fluid which is present in the lymphatic vessels and which may be collected by inserting a cannula into a lymphatic duct. From most regions of the body the lymph so collected is clear; but that from the intestines is generally opalescent or milky, depending on the amount of fat being absorbed at the time. The lymph forms part of the extracellular fluid, which is the immediate environment of the cells of the organism. For an understanding of the formation and of the physiological significance of lymph, therefore, it seems logical to consider first the basic structure of the extracellular phase of the body-fluids and the material exchanges that occur within it.

THE DISTRIBUTION OF FLUIDS IN THE BODY

More than half the mammalian organism is water, about 50 to 70 per cent of the body-weight in man (Soberman *et al.*, 1949; Pinson and Anderson, 1950; Steele *et al.*, 1950, McCance and Widdowson, 1951; Widdowson, McCance and Spray, 1951, Edelman *et al.*, 1952), and approximately the same proportion in other mammals (Harrison, Darrow and Yannet, 1936; Pace and Rathbun, 1945). In the newborn the figure is higher, but the proportion of water in the body-tissues thereafter decreases with age (Moulton, 1923; Barcroft, 1946, Friis-Hansen *et al*, 1951) The fairly wide variation observed in adult man and animals is due largely to the variable quantity of fat in the body. When allowance is made for body-fat which contains relatively little water, the body-water expressed as a percentage of the weight is remarkably constant, usually within the range of 70–75 per cent of the lean weight.

Extracellular and intracellular phases

The water which acts as a solvent for the various solutes may be either inside or outside the cells; but it is usually conceded to be freely diffusible throughout the body (cf Peters, 1935, 1944; Darrow and Yannet, 1935; Pinson, 1952), and so the osmotic pressure or the

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body, whereas the intracellular fluid contains most of the potassium All the fluid in the extracellular phase, including the lymph, has the same basic electrolyte framework.

In recent years experiments with radioactive isotopes have clearly shown that the water and the electrolytes of the body are not confined to the static anatomical compartments, but that a dynamic equilibrium exists between the various body-fluids. Water and electrolytes are moving constantly through the cell membrane, so that, although this membrane acts as a selective barrier, it is not impermeable to the electrolytes. To explain the movement of electrolytes through the cell membrane and at the same time the electrolyte gradients that exist between the intracellular and extracellular phases, various theories have been postulated (cf. Davson and Danielli, 1943; Höber, 1945; Ussing, 1949, Manery, 1954) It seems likely that there are pumping mechanisms associated with cell respiration and situated probably in the cell membrane, which actively pump such ions as sodium outwards from the interior of the cell. In recent years it has been suggested that water, too, may be actively transported outwards from the cell, if, as Opie (1949) has found, the cell contents are not isotonic but hypertonic compared with the extracellular fluid (Robinson, 1950, 1952, 1953; Robinson and McCance, 1952). The exact mechanism of these so-called "pumps" is still unknown, but it is sufficient here to recognize that the dynamic equilibrium between the electrolytes outside and inside the cell can be maintained only by the continual expenditure of energy by the cells.

Although the cell membrane does not maintain the various electrolytes in static compartments, it is, nevertheless, often of value in a consideration of the exchanges which take place between the cell and its environment to measure the volumes of extracellular and of intracellular water. The volume of extracellular water may be determined by estimating the dilution of a substance which, injected intravenously, will distribute itself equally throughout the extracellular phase. Unfortunately, no [illegible] volume of [illegible] stances [illegible] example, give measures of the sodium and chloride spaces which are not identical, because these ions are not wholly confined to the extracellular phase, but enter some of the cells, sodium more so than chloride (Amberson *et al.*, 1938; Manery and Hastings, 1939; Levitt and Gaudino, 1949, Danowski, 1951; Forbes and Perley, 1951; Deane and Smith, 1952, Deane, Ziff and Smith, 1952). The other substances used are in the main not normally present in the body, viz. thiocyanate, radiobromine (Br^{80}), inulin, sucrose, mannitol, sulphate and thiosulphate. Of all these substances, inulin gives the lowest figures and probably the

concentration of ions and molecules in the various fluids within the body must be fairly uniform. Actual osmotic equilibrium throughout the body-fluids will probably not be attained, but the movement of water is such that this equilibrium will always be approached.

While water may be freely diffusible, the cell membrane acts in some way as a selective barrier to the free movement of electrolytes, and the

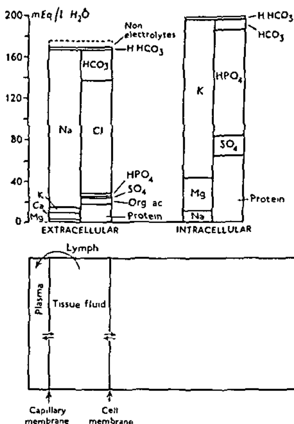


FIG 18.—Diagrammatic representation of the fluid and electrolyte distribution in the mammal
(Redrawn after Gamble, 1954)

distribution of the water will of necessity be related to the movement of these solutes. Morphologically, therefore, the body-fluids may be divided into two phases, the extracellular and the intracellular. In general this anatomical separation is in agreement with the electrolyte composition of the fluid in each of these phases (Manery and Hastings, 1939; Hastings, 1940–1941; Gamble, 1954). It is evident from Fig. 18 that the composition of these fluids is completely different. The extracellular fluid contains the greater part of the sodium and chloride in the

The extracellular phase contains further barriers which subdivide it and influence the movement of water and of other substances within it These barriers are cellular membranes and comprise the blood capillary membrane dividing the extracellular fluid into intravascular and extravascular compartments, the lymphatic capillary membrane separating the tissue fluid from the lymph and the serous membranes such as the pleura, pericardium and peritoneum. It is the exchange of materials through these membranes in the extracellular phase of the body-fluids that is of primary concern in the formation of lymph although the exchange across the cell membrane must also play a part.

Structure of the extracellular phase

Before discussing these exchanges, we should consider briefly the basic structure of the extracellular phase and in particular of the interstitial tissue or extravascular compartment, and our present conception of the state of the water in this phase. In the embryo, the forerunner of the connective tissues is a transparent, gelatinous, cell-free ground substance which is formed as a secretion of the cells of the various germ-layers. Fibrillation then occurs in this ground substance followed by invasion of the mesenchyme cells (Baitsell, 1924–1925). In later life this connective tissue consists of a semi-fluid gelatinous ground substance, which is colloidal in nature comprising mucopolysaccharide-protein complexes, and in which are embedded numerous collagen and elastic fibres (cf. Le Gros Clark, 1952; Ragan, 1952; Kellgren, 1952; Robb-Smith, 1954).

The fluid that is present in this tissue, the so-called tissue fluid or interstitial fluid, is not normally "free" in the sense that it is present in pools in lacunae. Clark and Clark (1933), by observations on the behaviour of minute quantities of substances injected and by the absence of Brownian movement which is always present in the lumen of stagnant vessels, concluded that in the tadpole's tail the tissue was clear and gelatinous and the water was not present in lacunae. In like manner, using the ear-chamber technique, these authors could not normally detect any free fluid in the tissue between the vessels in the skin of the rabbit's ear. It would seem that the water is associated with the colloid gel which makes up the "ground substance" of the connective tissue and varies in amount under different circumstances. Only when hydration of the gel is increased above a certain point can accumulations of "free" fluid in pools be observed.

In further investigations on the state of the interstitial fluid, McMaster and Parsons (1939*a* and *b*) observed the interstitial spread of vital dyes after injecting solutions of these dyes into the lymphatics in the tip of the mouse's ear. The dye first appeared outside the lymphatics as

losest approximation to the true anatomical extracellular space (cf. .evitt and Gaudino, 1950; Robinson and McCance, 1952). In Table 1 re the values found in the dog and in man by these different methods 'he figures are expressed as a percentage of the body-weight, without onsidering the fat content of the body.

TABLE 1

'olumes of distribution of substances used to measure extracellular fluid volume, s percentage of body-weight Each figure represents the mean of a group of individuals

	Dog	*Man*
nulin	20, 19	16, 15, 16
ucrose	—	20, 19
lannitol	—	23, 18, 16
'hloride	27, 23, 25	18
Bromide	31, 30	27, 23
ulphate	26	20, 24
odium	28, 30, 30	26, 26, 26
'hiocyanate	32, 30, 36, 32, 34	22, 23, 27, 25, 22, 24, 24
'hiosulphate	24, 27, 22	17, 18

From Levitt and Gaudino (1950), Cardozo and Edelman (1952)

While there are differences in the figures obtained by this wide variety f substances used for its determination, the total extracellular water may e taken, for practical purposes in discussing the fluid and material xchanges involved in the formation of lymph, as approximately 20–25 er cent of the body-weight, and the intracellular water will be the emainder, 45–50 per cent. This ratio of the two phases is not the same n all tissues. Table 2 shows that of the two soft tissues of greatest ulk in the body, muscle and skin, muscle has a high intracellular phase nd a low extracellular phase whereas the reverse holds for skin. In ther tissues these phases are more equally divided.

TABLE 2

Distribution of water in various tissues as percentage of tissue weight.

	Dog			*Monkey*			*Rabbit*		
	Total	*E.C.*	*I.C.*	*Total*	*E.C*	*I.C.*	*Total*	*E.C*	*I.C.*
Skin	55	46	9	70	59	11	71	45	26
Muscle	68	17	51	79	16	63	80	16	64
Liver	73	36	37	79	38	41	75	31	44
Skeleton	58	25	33	61	30	31	67	28	39
Intestines	69	38	31	80	39	41	80	37	43

From Harrison, Darrow and Yannet (1936)

of water which exist normally and to the "free" pools of water which are present in inflammatory oedema when the fibrous elements are separated.

The volume of extracellular water may therefore be regarded in part as "free" accumulations of water as in the blood plasma, the lymph, the cerebrospinal and ocular fluid, and any fluid in other cavities, and in part as "captured" water enveloping the connective tissue fibrils of the extravascular compartment or associated with the colloidal ground substance. Although the water may be "free" or "captured", there is a continuous interchange not only of water but of the various solutes between the different compartments of the extracellular phase. The life of the cell depends upon this movement of materials which after escaping from the blood vessels and passing among the cells return to the blood either directly through the capillary membrane or indirectly through the lymphatic vessels.

The Capillary Membrane

The exchange of greatest importance takes place through the capillary membrane which separates the rapidly circulating blood from the apparently stagnant interstitial fluid. In adult man the intravascular volume of 5 litres of blood, of which approximately 3 litres are plasma, circulates around the body about once a minute at rest, much more rapidly in exercise, and is separated from the 10 to 12 litres of tissue fluid by the thin capillary wall. In comparison with the rapid blood flow, the net filtration of fluid through this capillary wall is small. There is, however, a very rapid exchange of water and of dissolved substances by diffusion back and forth across the capillary membrane, and it is mainly by means of this "ultramicroscopic circulation" that materials are transported to and from the cells (cf Pappenheimer, 1953). This exchange of materials through the capillaries will depend primarily on the structure of their walls and on the volume and pressure of the blood flowing through them at the time.

Structure of the capillary membrane

Stock, 1944) Chambers and Zweifach (1947) have described on the inner side of the endothelium a non-cellular endocapillary lining which is possibly derived from the circulating blood proteins.

bristly, wavy lines of colour which could be bent and twisted by pressure with a micro probe and which would spring back to their original position when the pressure was removed, Fig. 19. These observations suggested that the dye moved between or along the connective tissue fibres which served as pathways for the transport of these molecules. These and other experiments on the movement of fluid in the interstitial tissue of the skin (McMaster, 1941) led to the belief that this fluid is normally

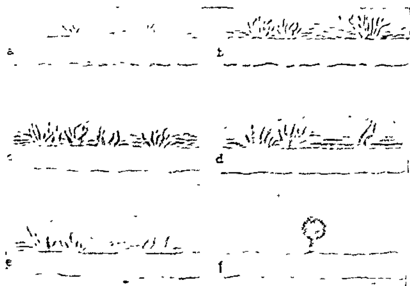


FIG 19—The outward movement of pontamine sky blue from the lymphatics of the ear of the mouse, and its subsequent movement into the normal connective tissue

First phase
(a) First appearance of coloured projections (2–7 min.)
(b) The projections become darker and longer (2–10 min)
(c) The projections become broader (5–12 min.)
Second phase
(d) Diffuse staining of the regions between the coloured projections
Third phase
(e) Diffuse blue staining easily displaced by pressure
Free fluid is now present in the tissues
(f) The appearance of dye that has escaped from a ruptured lymphatic There are no coloured projections

(From McMaster and Parsons, 1939)

in close association with the connective tissue fibrils. In contradistinction to these findings in normal tissues, "free" fluid in pools between the fibres was readily demonstrated when inflammatory or other oedema was produced

The evidence indicates, therefore, that normally only submicroscopic quantities of fluid can be "free" in the interstitial spaces. It is possible that fluid movement through the connective tissues may take place between or surrounding the connective tissue fibres in thin films by surface forces. McMaster and Parsons refer to the "captured" films

the capillaries. How the walls of the capillaries contract and relax independently of the arterioles has been the subject of much controversy, some investigators maintaining that the narrowing of the lumen is due to the contraction of cells surrounding the endothelium (cf. Rouget, 1873; Krogh, 1929). Clark and Clark (1925, 1931, 1939), in studying the growth of blood capillaries in the tadpole's tail and in the rabbit's ear, observed cells resembling fibroblasts adhering to the walls of newly formed capillaries, but contractility of these adventitial cells was never seen. Nor did they observe active contractility in any capillary composed of simple endothelium in contrast to those small vessels with muscle cells in their walls. They concluded that active contractility of true capillaries, if present at all, is so slight as to be negligible as a factor in control of circulation in the rabbit ear preparation. Other observers have described the narrowing or obliteration of the capillary lumen by a swelling of the endothelial nuclei (Steinach and Kahn, 1903; Kahn and Pollak, 1931; Sanders, Ebert and Florey, 1940); but Clark and Clark (1943), Chambers (1946) and Taylor (1953) attribute this nuclear swelling to the release of tension on the endothelial cell

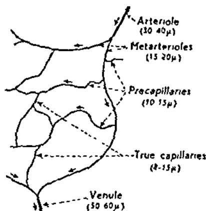


FIG 21 —Diagrammatic representation of the capillary bed of the omentum (From Zweifach, Lee, Hyman, and Chambers 1944)

In recent years Chambers and Zweifach (1944, 1947) from studies on mammalian as well as amphibian capillaries have put forward their concept of the continually changing flow pattern and pressure in a group of capillaries. They have shown in a number of tissues that the peripheral vascular bed consists of a group of functioning units, each unit comprising a central, capillary-like channel of which the true capillaries are side branches, Fig. 21. The proximal or contractile portion of this central or a-v channel is encircled with single discontinuously arranged muscle cells and is termed the metarteriole, whereas the venular end is devoid of muscle cells. The true capillaries leave the metarteriole and reunite with the a-v through-capillary at the venular end. The junction of the true capillary with the metarteriole is encircled with contractile muscle cells and is called the pre-capillary sphincter. The metarteriole and the pre-capillary sphincters undergo spontaneous periodic constriction and relaxation which Chambers and Zweifach call vasomotion. Normally, the blood-flow through the a-v channel may be fairly constant,

The essential component of the capillary membrane is the endothelium The boundaries of the endothelial cells can be shown histologically in most capillaries by staining with silver salts. In the growing ends of capillaries in the rabbit ear chamber preparation, however, Clark and Clark (1939) failed to outline the cells with silver and so concluded that here the capillary membrane is a syncytium. In considering the permeability of the fully formed capillary, we should regard the wall as consisting in the main of a plasma membrane (cf. Davson and Danielli, 1943; Hober, 1945) which envelops the protoplasm of the endothelial cells, and to a lesser extent of specialized regions. The plasma membrane

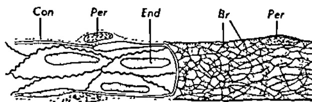


FIG 20—Diagram of the wall of a capillary
Br, branches of pericyte, *Con*, connective tissue, *End*, endothelial nucleus, *Per*, nucleus of pericyte
(From Danielli and Stock, 1944)

of the cells is believed to have a low permeability to ions and lipid-insoluble molecules but a high permeability to oxygen, carbon dioxide and other lipid-soluble substances. On the other hand, the specialized regions, which Chambers and Zweifach (1940, 1947) believe to be the intercellular cement, render the capillary wall as a whole highly permeable to water, ions and other lipid-insoluble materials. As we shall see later in this chapter the permeability of the capillary membrane to lipid-insoluble molecules depends upon the size of the molecules, but is otherwise non-selective, and the exchange of substances through this membrane does not involve the endothelial cells in an expenditure of energy. These experimental findings which are generally accepted suggest that the permeability depends primarily upon the specialized areas which represent only a small fraction of the total area of the membrane.

Architecture of the capillary bed

It has long been recognized that the flow and pressure in individual capillaries varies from time to time (cf. Dale, 1920, Richards and Schmidt, 1924; Lewis, 1927; Krogh, 1929; Clark and Clark, 1931). The flow may cease in a capillary following the contraction of the arteriole supplying it. In addition to this coarse adjustment of the blood supply to a region, there is also a fine adjustment depending on the varying needs of small areas of tissue and carried out by the changing tone of

water, 60 per cent of plasma sodium and 60 per cent of the plasma chloride were exchanged per minute and in man 105 per cent of plasma water and 78 per cent of plasma sodium were exchanged per minute with extravascular water and sodium (Flexner, Gellhorn and Merrell, 1942; Merrell, Gellhorn and Flexner, 1944; Flexner, Cowie and Vosburgh, 1948) These observations indicate the very rapid exchange of materials by diffusion through the capillary membrane. Pappenheimer, Renkin and Borrero (1951) using a different technique, the perfused hind limb preparation, calculated that the diffusion of water, sodium chloride, urea and glucose in both directions through the capillary wall to be respectively 80, 40, 30 and 10 times the rates at which these substances were brought to the tissues by the incoming blood, i.e. at a very much greater rate than that found by Flexner and his colleagues.

All the evidence suggests that although the diffusion of small ions and lipid-insoluble molecules is very rapid, these substances do not diffuse at the same rate, but at rates depending on the size of the ion or molecule (Starling, 1896; Keys, 1937; Flexner, Cowie and Vosburgh, 1948; Pappenheimer, 1953). By this means of exchange through the capillary membrane, the bulk of the transport of materials to and from the cells of the organism occurs.

The facts that these substances pass through the capillary wall without any energy transformations by the endothelial cells, that the rate of diffusion depends upon the size of the ion or molecule and that, as we shall see, the rate of net fluid movement across the capillary wall, one way or the other, depends merely upon the balance between the hydrostatic and osmotic pressures have led to the "Pore Theory" of capillary exchange. This theory postulates that the capillary membrane contains numerous pores or ultramicroscopic openings which allow the rapid passage of small substances, although at different rates, but which in general prevent the free movement of the larger protein molecules. Pappenheimer has calculated that uniform cylindrical pores with a diameter of 60 to 90 Å and a population density of $1-2 \times 10^{9}$ per $cm.^2$ of capillary wall would account for the diffusion rates he observed in his experiments. The different diffusion rates of molecules or ions through pores which are commensurate with the size of the diffusion particles have been explained by the phenomenon of "restricted diffusion".

The situation of these pores in the capillary membrane is not certain. Chambers and Zweifach (1940, 1947) have put forward the concept that they are present in the intercellular cement which comprises only one per cent of the total area of the capillary wall. They postulate that the intercellular cement is being continually formed by the endothelial cells and that the endocapillary lining extends into the pores and regulates their size. Flexner and his colleagues concluded that diffusion was too

but the vasomotion of the pre-capillary sphincters alters the flow and pressure in these capillaries from moment to moment. The vasomotion is probably affected by the local metabolism. Although this pattern of capillary flow has been shown to exist in several tissues, Grafflin and Bagley (1952) have described different capillary patterns in other tissues. Some tissues, too, such as the liver and spleen have specialized vascular arrangements which will be considered in Chapter 3 with the lymph flow from these organs.

Whatever the architecture of the capillary bed in any tissue and whatever the true mechanism of capillary contraction may be, there is no doubt that the flow of blood through a given capillary bed is continually changing. This alteration from moment to moment not only of the blood flow but also of the pressure in the capillaries must play an important rôle in the material exchanges between the blood and the extravascular fluids.

Exchange of materials through the capillary membrane

The composition and volume of the extravascular or tissue fluid may be affected by exchanges with the plasma through the capillary wall on the one hand and by exchanges with intracellular materials through the cell membrane on the other. Although the changing metabolism of cells must affect the composition of the tissue fluid of the region concerned, *it is the exchanges through the capillary membrane that have been most* extensively studied and which are largely concerned with lymph formation.

This exchange of materials between the blood and the extravascular fluid is by diffusion or by bulk filtration. Diffusion may occur even against the flow by filtration. For example, if a hypertonic solution of sodium chloride or of glucose is injected intravenously, water will be withdrawn from the extravascular spaces into the capillary, diluting the blood, while at the same time the injected material will diffuse outward against the bulk movement of fluid. In normal circumstances there is an extremely rapid diffusion of all small lipid-insoluble ions and molecules back and forth through the capillary membrane. This has been determined by the use of tracer amounts of radioactive isotopes such as Na^{24} without upsetting normal plasma levels or electrolyte and water balance. Hevesy and Jacobsen (1940) and Hahn and Hevesy (1940) injected intravenously into rabbits heavy water, Na^{24}, Cl^{38}, K^{42} and Br^{80} and demonstrated very rapid diffusion through the capillary wall. Water penetrated most rapidly and became diluted with a volume equal to that of the extracellular water within 30 seconds. Of the electrolytes, potassium diffused most rapidly, while sodium, chloride and bromide reached an equilibrium at about the same time. Flexner and his colleagues by similar methods found that in the guinea-pig 140 per cent of the plasma

water, 60 per cent of plasma sodium and 60 per cent of the plasma chloride were exchanged per minute and in man 105 per cent of plasma water and 78 per cent of plasma sodium were exchanged per minute with extravascular water and sodium (Flexner, Gellhorn and Merrell, 1942; Merrell, Gellborn and Flexner, 1944; Flexner, Cowie and Vosburgh, 1948). These observations indicate the very rapid exchange of [illegible] urea and glucose in both directions through the capillary wall to be respectively 80, 40, 30 and 10 times the rates at which these substances were brought to the tissues by the incoming blood, i.e. at a very much greater rate than that found by Flexner and his colleagues.

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rapid to accept the hypothesis that water as well as sodium and chloride ions passed only through an area equal to that of the intercellular cement. *Pappenheimer, however, has calculated that the total cross-sectional area* of the pores comprises less than 0·2 per cent of the capillary wall, so that it would seem possible that these pores are confined to the intercellular cement.

In contradistinction to exchange by diffusion, exchange by net filtration of fluid is small, amounting in all, in both directions, to about 2 per cent only of the plasma flow. In general this filtration depends upon the balance between the capillary pressure and the effective osmotic pressure of the plasma proteins. We have seen earlier how the concentration of plasma proteins in the animal kingdom increased with the increase in blood pressure. In this way the circulation of the blood could be enormously increased with a commensurate increase in the exchange of materials by diffusion, without the loss of much fluid by filtration into the interstitial spaces. The object of the balance of these pressures is to reduce net filtration of fluid to a minimum, but at the same time to allow maximum exchange by diffusion between the blood and extravascular fluid.

Starling (1896) first clearly outlined the factors responsible for this balance of fluid exchange through the capillary membrane. In what has become known as the "Starling Hypothesis", he maintained that the direction and rate of fluid transfer was proportional to the algebraic sum of the effective hydrostatic pressure in the capillaries and the osmotic pressure of the plasma proteins. For this theory to operate, he pointed out that although the capillary membrane was freely permeable to crystalloids, it was relatively impermeable to the larger plasma protein molecules Describing how isotonic salt solutions were absorbed from the tissues into the blood vessels, he says: "I believe the explanation is to be found in a property on which much stress was laid by the older physiologists, and which they termed the high endosmotic equivalent of albumen. It must be remembered that the earlier workers used animal membranes in their experiments on osmotic interchanges. These membranes permit the passage of water and salts, but hinder the passage of coagulable proteid. Whereas the enormous pressures of the salts and crystalloids in the various fluids of the body are of very little importance for the function of absorption by the blood vessels, the comparatively insignificant osmotic pressure of the albumens is of great importance." He found by estimation that the osmotic pressure of the plasma proteins was 30 to 40 mm. Hg and he continues: "The importance of these measurements lies in the fact that, although the osmotic pressure of the proteids of the plasma is so insignificant, it is of an order of magnitude comparable to that of the capillary pressures; and whereas capillary

pressure determines transudations the osmotic pressure of the proteids of the serum determines absorption. Moreover, if we leave the functional resistance of the capillary wall to the fluid through it out of account, the osmotic attraction of the serum for the extravascular fluid will be proportional to the force expended in the production of this latter, so that, at any given time, there must be a balance between the hydrostatic pressure of the blood in the capillaries and the osmotic attraction of the blood for the surrounding fluids. With increased capillary pressure there must be increased transudation until equilibrium is established at a somewhat higher point, when there is a more dilute fluid in the tissue spaces and therefore a higher absorbing force to balance the increased capillary pressure. With diminished capillary pressure there will be an osmotic absorption of salt solution from the extravascular fluid, until this becomes richer in proteids; and the difference between its (proteid) osmotic pressure and that of the intravascular plasma is equal to the diminished capillary pressure."

Factors concerned in filtration

Starling's hypothesis has since been confirmed by direct measurements of the pressures concerned and observations on the movement of fluid. If the theory is correct, the same degree of filtration should occur with the same driving force irrespective of the actual value of each of the opposing pressures; that is, a rise in capillary pressure or an equivalent fall in plasma osmotic pressure will result in the same increase in filtration rate.

Landis (1927) by micro-injection on single capillaries in the frog's mesentery confirmed this by direct measurement. He found that when the capillary pressure was less than 9 cm. water fluid moved into the blood and when it was greater than 12·5 cm. water fluid moved into the tissue spaces. Using a mammalian hind-leg preparation, Pappenheimer and Soto-Rivera (1948) showed that the mean pressure head available for net fluid transfer across the capillary membrane is the mean capillary pressure minus the effective osmotic pressure of the plasma proteins. They further showed that the rate of filtration or absorption was proportional to this pressure head irrespective of the absolute values of each, over a wide range of values, Fig. 22.

Indirectly, without actual measurement of the pressures concerned, the evidence all supports the Starling Hypothesis. Raising the capillary pressure by increasing the venous pressure, Fig 23, or by arteriolar dilatation as in warming a limb, leads to an increase in the rate of capillary filtration (Landis *et al*, 1932; Krogh, Landis and Turner, 1932; Landis and Gibbon, 1933; White, Field and Drinker, 1933; Courtice, 1946). When the plasma protein concentration is increased by the intravenous

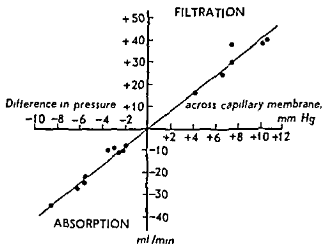


FIG 22—Fluid exchange in the perfused hind leg of a cat

The rate of fluid exchange is proportional to the difference between the mean hydrostatic pressure in the capillaries and the sum of all pressures opposing filtration and is independent of the absolute values of these quantities

(Redrawn from Pappenheimer and Soto-Rivera, 1948)

injection of concentrated albumin solution, tissue fluid is withdrawn into the blood stream and haemodilution occurs (Heyl, Gibson and Janeway, 1943) A fall in the plasma proteins leads to an increased filtration rate

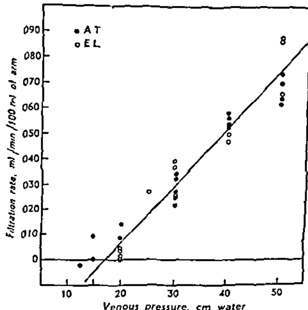


FIG 23—Chart showing the relation between fluid filtration and venous pressure in man

Observations made in two subjects ● A T, O E L

(From Krogh, Landis and Turner, 1932)

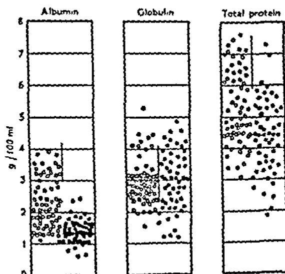


Fig 24.—The relation between the plasma protein concentration and oedema in dogs
○ No oedema ● Oedema
(From Weech, Snelling and Goettsch 1933)

and ultimately to visible oedema as in malnutrition and plasmaphaeresis (Kobman, 1920; Field and Drinker, 1931*b*; Shelburne and Egloff, 1931, Darrow, Hopper and Cary, 1932, Weech, Snelling and Goettsch, 1933).

The relation between the plasma protein concentration and oedema in dogs is shown in Fig. 24. This indicates that oedema assessed by finger palpation is related to the plasma albumin and not the globulin level. When the albumin level is less than 1 g per cent oedema always occurs, but when the albumin is above 2 g per cent oedema is rarely present. From Fig 25 it is evident that oedema occurs more readily in malnutrition than in plasmaphaeresis. Weech, Snelling and Goettsch have suggested that this may be due to the loss of elasticity of the connective tissues in malnutrition. The tissues would thus

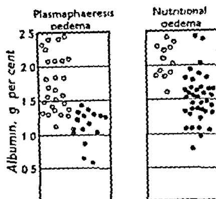


Fig 25.—The relation between plasma albumin and oedema following plasmaphaeresis and malnutrition
○ No oedema ● Oedema
(From Weech, Snelling and Goettsch, 1933)

accommodate more fluid before tissue pressure rose sufficiently to balance the lowered osmotic pressure of the plasma proteins. Although visible oedema is observed at fairly definite low levels of albumin, the evidence favours the view that as the plasma albumin concentration falls there is a gradual, rather than an abrupt, fluid accumulation in the tissues.

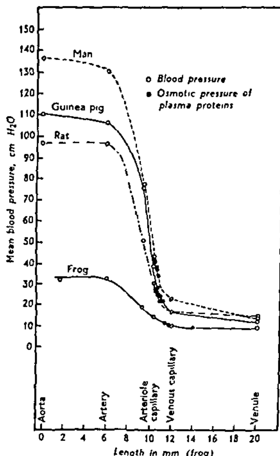


FIG 20 —The pressure in different vessels of the vascular tree and the osmotic pressure of the plasma proteins in man, guinea-pig, rat and frog
(From Landis, 1934)

The capillary pressure. Of the pressures concerned in filtration the capillary hydrostatic pressure is very variable whereas the osmotic pressure of the plasma proteins normally remains fairly constant. The pressure within a single capillary varies, not only from time to time with changes in arteriolar tone and in vasomotion of the precapillary sphincters, but also at any one time from one end to the other. This gradient of pressure from the arteriolar to the venular end of a capillary has been measured

directly in the frog's mesentery (Landis, 1925–1926), web and muscle of frog (Landis, 1931), mesentery of the rat and guinea-pig (Landis, 1930*a*) and in the skin of man (Landis, 1930*b*). The average fall in pres[illegible] throughout the vascular tree in these animals may be represent[illegible]

[illegible] osmotic pressure of the proteins, [illegible] of filtration pressure at the arteriolar end of the capillary, [illegible] re favouring absorption at the venular end.

TABLE 3

Blood colloid osmotic pressure and capillary pressure (micro-injection determinations) in cm water

Animal	*Colloid osmotic pressure of blood*	*Arteriolar capillary pressure*	*Venous capillary pressure*	*Tissue*
Frog . . .	11·7	14·4	10·1	Mesentery
		14 9	9 5	Skin
		14 5	10 0	Mesentery
Rat .	22 0–26 5	30 0	17 0	Mesentery
Guinea-pig .	22 5–27 5	38 5	17 0	Mesentery
Man . .	28 5–40 0	43 5	16 5	Skin, base of nail

Modified from Landis (1934)

Although this represents the average conditions that occur in a capillary in standard conditions, it can readily be conceived that under certain conditions filtration will occur along the whole length of a capillary; on the other hand under other circumstances reabsorption may take place along the whole length of a capillary. Posture, for example, greatly affects capillary pressure In the upright position the capillary pressure in the dependent limbs greatly increases with an overall increase in filtration leading to excess tissue fluid in the limbs and concentration of the blood cells and protein (Thompson, Thompson, Dailey, 1928; Waterfield, 1931*a* and *b*; Keys and Taylor, 1935*b*). In contradistinction to this increase in pressure, the capillary pressure in large areas decreases after haemorrhage leading to bulk reabsorption of fluid into the blood stream with haemodilution.

Colloid osmotic pressure The osmotic pressure of the plasma proteins [illegible] level of the albumin. Measurements of [illegible] by Keys and Taylor (1935*a*) [illegible] re and the level of total protein and albumin has been expressed [illegible] ormula $P = C(21\ 4 + 5\ 9A)$, where P is the osmotic pressure in millimetres of

accommodate more fluid before tissue pressure rose sufficiently to balance the lowered osmotic pressure of the plasma proteins. Although visible oedema is observed at fairly definite low levels of albumin, the evidence favours the view that as the plasma albumin concentration falls there is a gradual, rather than an abrupt, fluid accumulation in the tissues.

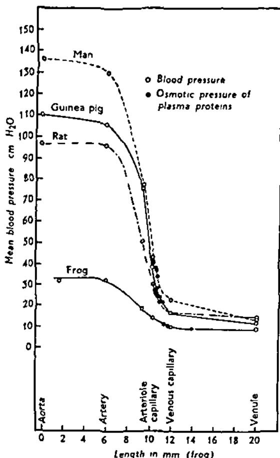


FIG 26 —The pressure in different vessels of the vascular tree and the osmotic pressure of the plasma proteins in man, guinea-pig, rat and frog
(From Landis, 1934)

The capillary pressure. Of the pressures concerned in filtration the capillary hydrostatic pressure is very variable whereas the osmotic pressure of the plasma proteins normally remains fairly constant. The pressure within a single capillary varies, not only from time to time with changes in arteriolar tone and in vasomotion of the precapillary sphincters, but also at any one time from one end to the other. This gradient of pressure from the arteriolar to the venular end of a capillary has been measured

directly in the frog's mesentery (Landis, 1925–1926), web and muscle of frog (Landis, 1931), mesentery of the rat and guinea-pig (Landis, 1930*a*) and in the skin of man (Landis, 1930*b*). The average fall in pressure throughout the vascular tree in these animals may be represented as in Fig. 26

When the capillary pressure is considered with the opposing osmotic pressure of the proteins, it is seen in Table 3 that there is a head of filtration pressure at the arteriolar end of the capillary, but a pressure favouring absorption at the venular end.

TABLE 3

Blood colloid osmotic pressure and capillary pressure (micro-injection determinations) in cm. water.

Animal	*Colloid osmotic pressure of blood*	*Arteriolar capillary pressure*	*Venous capillary pressure*	*Tissue*
Frog . . .	11·7	14 4	10·1	Mesentery
		14 9	9 5	Skin
		14 5	10 0	Mesentery
Rat . . .	22·0–26 5	30 0	17 0	Mesentery
Guinea-pig	22 5–27 5	38 5	17·0	Mesentery
Man . . .	28 5–40 0	43 5	16 5	Skin, base of nail

Modified from Landis (1934)

Although this represents the average conditions that occur in a capillary in standard conditions, it can readily be conceived that under certain conditions filtration will occur along the whole length of a capillary; on the other hand under other circumstances reabsorption may take place along the whole length of a capillary. Posture, for example, greatly affects capillary pressure. In the upright position the capillary pressure in the dependent limbs greatly increases with an overall increase in filtration leading to excess tissue fluid in the limbs and concentration of the blood cells and protein (Thompson, Thompson, Dailey, 1928; Waterfield, 1931*a* and *b*; Keys and Taylor, 1935*b*) In contradistinction to this increase in pressure, the capillary pressure in large areas decreases after haemorrhage leading to bulk reabsorption of fluid into the blood stream with haemodilution.

Colloid osmotic pressure. The osmotic pressure of the plasma proteins is largely dependent upon the level of the albumin. Measurements of this pressure may be made by an apparatus similar to that used by Keys and Taylor (1935*a*). The relation between the osmotic pressure and the level of total protein and albumin has been expressed by the formula $P = C(21{\cdot}4 + 5\ 9A)$, where P is the osmotic pressure in millimetres of

water, C is the total protein concentration and A is the albumin concentration in grams per 100 ml. (Wells, Youmans and Miller, 1933). Determinations of the colloid osmotic pressure of the plasma of different animals are given in Table 4.

TABLE 4

Colloid osmotic pressure and protein concentration in serum or plasma of different species.

Animal		*Protein concentration g %*	*Osmotic pressure cm H_2O*	*Author*
Toadfish	(10)	4·17 (3·25–5·29)	10·4 (7·6–12·7)	Turner (1937)
Wolf fish	(8)	4·61 (3·16–6·52)	15·3 (11·9–17·8)	do
Turtle	(16)	3·55 (2·51–4·21)	7·4 (3·9–11·2)	Campbell and Turner (1937)
Frog	(12)	1·98 (1·4–4·29)	5·3 (1·5–13·4)	Churchill, Nakazawa and Drinker (1927)
Nocturus	(18)	2·24 (1·24–3·50)	9·5 (5·0–15·8)	White (1928)
Rabbit	(24)	6·20 (5·7–7·1)	28·7 (25·9–33·0)	Tada and Nakazawa (1930)
"	(34)	5·99 (4·99–6·68)	29·5 (22·4–35·8)	Izumi (1933)
Dog	(16)	7·50 (6·70–9·80)	28·2 (24·5–36·5)	Kimura and Nakazawa (1930)
"	(18)	6·04 (4·65–7·12)	34·0 (18·2–47·8)	Wells (1932*a* and *b*)
"	(13)	6·25 (5·62–7·64)	30·6 (22·2–36·9)	Field *et al* (1934–5)
Cat	(5)	7·4 (6·6–8·4)	31·0 (26·0–34·5)	Krogh (1929)
Man	(38)	8·94 (6·5–10·3)	36·9 (20·3–43·4)	Serr (1924)
"	(8)	7·56 (7·2–8·6)	36·2 (32·5–40·1)	Iversen and Nakazawa (1927)
"	(34)	8·31 (6·9–10·0)	38·5 (31·5–46·5)	Ito, Seki and Nakazawa (1929–30)
"	(23)	7·75 (6·55–9·35)	36·0 (32·6–38·3)	Kylin (1931)

Abridged table from Drinker and Yoffey (1941)

Figures in brackets represent number of individuals in group and the ranges of the protein concentration and osmotic pressure

It has been shown that filtration through the capillary membrane depends upon the balance between the capillary pressure and the osmotic pressure of the proteins. Since, as we shall see later, some protein escapes into the extravascular fluid, the effective osmotic pressure of the proteins will be determined by the difference in the protein concentrations in the plasma and in the extravascular fluid immediately outside the capillary wall. Although the level of protein in the capillaries may easily be determined, that in the tissue fluid cannot. The relation between the concentration of protein in the lymph and that in the tissue fluid will be discussed later in this chapter.

Tissue pressure If the capillary pressure and the colloid osmotic pressure were the only forces involved in the filtration of fluid across the capillary membrane, fluid would accumulate in progressively increasing quantities in the dependent tissues in the erect posture or in other tissues with a moderate increase in venous pressure. Landis and Gibbon (1933) showed by means of a pressure plethysmograph that in the human forearm the rate of filtration produced by any given venous pressure

decreased as fluid accumulated in the tissues, and that when an appreciable amount of fluid had accumulated low filtering heads of pressure, which originally produced filtration, now failed to do so. These observations indicated that when fluid accumulates in the tissue spaces a tension develops which opposes filtration. Landis and Gibbon estimated by this method that with large accumulations of fluid in the tissue spaces the filtration rate was decreased by an amount which was equivalent to a tissue pressure of about 35 cm. water.

The tissue tension has also been determined directly Burch and Sodeman (1937, 1939) and Wells, Youmans and Miller (1938) measured subcutaneous, intracutaneous and intramuscular pressures in man. On the dorsum of the hand the normal tissue pressure in one group of subjects was 1·8 to 5·4 cm. water with a mean of 2 9 cm. During prolonged venous congestion the tissue pressure in those leg muscles which are tightly covered with fascia rose to 50 cm. water or higher, which was probably high enough to stop filtration, whereas in the gastrocnemius which is loosely covered the pressure did not rise above 20 cm. water during congestion. McMaster (1946*a*) used a technique in which practically no fluid entered the tissue so as to minimize any error due to the introduction of appreciable quantities of fluid. In the skin of the ears, back and thighs of the mouse, readings fell within the range of 0·5 to 5·0 cm. water with an average of 1·7, whereas in oedema due to heat or the application of xylol the pressure rose to 4·0 to 9·2 cm water. In man in the skin of the volar surface of the forearm and the dorsum of the ankle with the patient lying flat, the pressure averaged about 3 1 cm. water while in the tense skin over the deltoid muscle the tension was 5 to 6 7 cm. water. *After venous obstruction (McMaster, 1946b) the* interstitial pressure rose in the skin of the mouse's leg from a normal range of 2 6–4·2 to about 32 cm. water, thereafter remaining constant. It seems, therefore, that at this pressure the escape of fluid is checked. When the veins of the arm and leg of man are obstructed the pressure rises from 2·5–3·5 to 15 0–23 0 cm. water. In all these observations the increase in tissue pressure follows an acute increase in the quantity of tissue fluid After a time, however, when the stretched connective tissue fibres lose their elasticity, the tissue pressure falls.

It is evident from such measurements that as soon as the amount of tissue fluid increases, the tension created in the tissue spaces outside the capillary increases to oppose further filtration This tissue tension has to be considered with the capillary and osmotic pressure of the proteins in evaluating the factors concerned in the fluid balance across the capillary membrane. The tissue tension will also be affected by muscular contraction. The intramuscular pressure may rise very considerably during voluntary contraction. Wells *et al* (1938) found that

during maximal voluntary contractions the intramuscular pressure rose to values of 10 to 118 cm. water depending upon the tightness of the fascia.

The Formation of Lymph

The lymphatic capillaries are sometimes closely associated with the small blood vessels with virtually nothing between the two membranes while in other cases they bear no relation to them (Clark and Clark, 1937). In any region the fluid that enters the lymphatic capillaries to become lymph, therefore, may be that which is adjacent to the arteriolar end of a blood capillary or to the venular end, or it may be the fluid that is relatively distant from a blood capillary. McMaster (1947) investigated the relative pressures within the cutaneous lymphatic capillaries and in the surrounding tissues in the mouse's ear. In a group of experiments he found that the mean intralymphatic pressure was 1·2 cm. water and the interstitial pressure 1·9 cm. water. There was always a gradient of pressure from the interstitial tissue to the lumen of the lymphatic and during oedema caused by mild heat or xylol this gradient was increased even though the intralymphatic capillary pressure was increased. These findings show that a pressure gradient is an important factor in lymph formation both under normal and pathological conditions, the lymphatic capillary being prevented from collapsing by the mechanism already described (see p. 20)

In normal tissues the lymphatics drain any fluid in excess of filtration over reabsorption through the capillary wall. As the formation of tissue fluid increases, the tissue pressure rises, the lymphatic vessels dilate and the formation of lymph increases. This is well shown in experiments where the filtration rate is increased by increasing the filtering pressure head or by altering the permeability of the membrane. For example, the flow from a cannula in the main lymphatic trunk draining the paw of a dog increased when the net filtration was increased by warming the limb in a water bath (Courtice, 1946). In other experiments in which net filtration was increased by intravenous transfusions (Reinhardt, 1952; Korner, Morris and Courtice, 1954) or by venous obstruction (Starling, 1894; Field and Drinker, 1931*b*), the lymph flow was also increased, as shown in Fig. 27 and Table 5.

Besides removing the excess tissue fluid, so that normally there is no appreciable amount of "free" fluid in the tissues, the lymphatic vessels have the special function of absorbing protein. It is now generally agreed that extra-vascular protein is all, or almost all, returned to the blood stream by the lymphatic system. Although it was earlier known that lymph from some regions of the body contained protein (cf. Drinker and Field, 1931), Drinker and his colleagues were the first to emphasize

the importance of the escape of protein from practically all blood capillaries and its return via the lymphatics. Drinker and Field collected lymph from the cervical, leg, and renal lymphatics as well as from the thoracic duct and found considerable concentrations of protein *in all* samples. They concluded from these experiments "that the capillaries

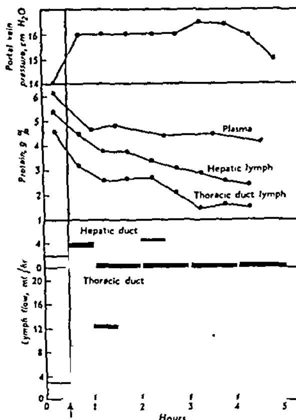


FIG 27 —The effect of the intravenous infusion of Ringer-Locke Solution (30 ml /kg /hr) on the flow and protein concentration of hepatic and thoracic duct lymph and on the portal pressure in the cat

(By courtesy of Mr Bede Morris)

practically universally leak protein; that this protein does not re-enter the blood vessels unless delivered by the lymphatic system; that the filtrate from the blood capillaries to the tissue spaces contains water, salts and sugar in concentrations found in blood, together with serum globulin, serum albumin and fibrinogen in low concentration, lower probably than that of tissue fluid or lymph; that water and salts are reabsorbed by blood vessels and protein enters the lymphatics together

during maximal voluntary contractions the intramuscular pressure rose to values of *10 to 118 cm. water depending upon the tightness of the* fascia.

The Formation of Lymph

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peritoneally. Barnes and Trueta (1941) injected snake venom into the legs of rabbits after severing all the lymphatics and found that they survived whereas intact animals died This suggests that the larger protein molecules are absorbed mainly or probably wholly by the lymphatics.

Courtice and his colleagues labelled plasma protein with the dye T1824, and found that when protein labelled in this way was introduced into the pleural or peritoneal cavities, it was all or nearly all absorbed by the lymphatics (Courtice and Simmonds, 1949; Courtice and Steinbeck, 1950, 1951). This label has been shown to be a very firm one (Rawson, 1938; Allen and Orahovats, 1951) and proteins labelled in this way behave as unlabelled proteins as far as the rate of absorption is concerned. The advantage of labelling the proteins is that homologous plasma proteins or the animal's own protein can be used. This may be important when assessing the function of the lymphatic system in returning to the blood stream those proteins which have escaped through the capillary membrane. In these experiments, as well as those of Lewis and of Field and Drinker, a small percentage of the absorbed dye generally entered the blood stream by some undetectable route. This could be by small subsidiary entrances into the venous system of lymphatics which had not been tied off or by diffusion directly into the blood capillaries. As it is impossible to be absolutely sure that there is not a small branch duct entering the vein in the neck or some other vein as described in Chapter 1, it is not possible to be sure whether or not a small amount of protein can or does pass back through the blood capillary membrane. In any case this is at most only a small amount as shown in Fig. 28.

Protein does not readily re-enter the blood capillaries directly because the pressures are not favourable for such a backward filtration. Diffusion must play a negligible part for even when high concentrations of protein are introduced into the peritoneal cavities, to give a high protein concentration gradient from tissue fluid to plasma, there is no appreciable absorption of protein by the blood capillaries

We see, therefore, that although the protein molecules do not re-enter the blood capillaries directly, they very readily enter the lymphatic vessels. There is thus set up an extravascular circulation of the plasma proteins and the main function of the lymphatics is this return to the blood stream of the protein that has escaped from the blood vessels Should the lymphatics become blocked as in filariasis or by experimental injection of a sclerosing agent into the vessels, elephantiasis will result. Drinker, Field and Homans (1934) produced blockage of the lymphatics of the leg of the dog by injecting into the cannulated lymphatics, crystalline silica and quinine hydrochloride. After repeated injections the lymphatic drainage of the hind leg became permanently impassable There resulted

TABLE 5

Effect of increase in venous pressure on flow and protein concentration of lymph from the fore-leg of a dog.

Time	*Pressure in cuff* mm Hg	*Venous pressure* mm Hg	*Lymph flow per 10 minutes* ml	*Protein concentration* %	*Remarks*
10.50–11.00	0	14	0·07	0·77	Dog sat on haunches and stood
11.00		14		0·77	
11.00–11.10	0	14	0·15	0·67	Walked a few steps and stood
11.10–11.20	0	14	0·10	0·63	Standing only
11.20–11.30	0	14	0·14	0·62	Standing and lying down
11.30–11.40	0	14	0·10	0·57	Standing and lying down
11.40					Blood pressure cuff on and pressure raised to 20 mm Hg
11.40–11.50	20	26	0·15	0·57	Standing only
11.50–12.00	20	26	0·15	0·52	Standing only
12.00–12.10	20	26	0·23	0·45	Standing. Moved slightly
12.10–12.25	0	14			Cuff pressure released. Dog stood
12.25–12.35	20	26	0·16	0·35	Standing only
12.35–12.45	20	26	0·14	0·33	Standing only
12.45–12.55	20	26	0·36	0·32	Moved slightly. Standing
12.55–1.05	20	26	0·32	0·29	Moved slightly. Standing
1.05–1.10	20	26			4 ml novocaine, adrenin solution infiltrated around wound
1.10–1.20	20	26	0·70	0·24	Taking a few steps
1.20–1.30	60	45	0·54	0·21	Standing only
1.30–1.55					Pressure released. Dog taken for a walk out of doors
1.55–2.05	40	34	0·29	0·29	Standing quietly
2.05–2.15	40	34	0·26	0·21	Standing quietly
2.15–2.25	40	34	0·36	0·21	Standing quietly
2.25–2.35	40	34	0·49	0·21	Standing quietly
2.40	60	45			
2.40–2.50	60	45	0·49	0·21	Dog lying down and standing
2.50–3.00	60	45	0·47	0·21	Dog lying down and standing

From White, Field and Drinker, (1933)

with water and salts in the concentration existing in the tissue fluid at the moment of lymphatic entrance".

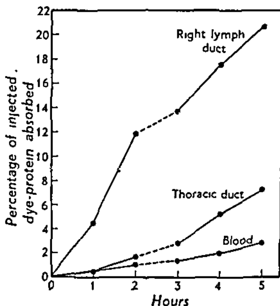
The function of the lymphatics in returning extravascular protein to the blood stream has been demonstrated in several ways. Conklin (1930) injected foreign proteins into the circulating blood of the frog and found that they passed through the skin capillaries and were recovered in the lymph. When the proteins were injected into the lymph spaces, however, there was no direct absorption into the blood. Lewis (1921) injected horse serum subcutaneously into dogs and detected it in thoracic duct lymph in 40 minutes and in blood in 3½ hours. Field and Drinker (1931*a*) also demonstrated that horse or dog serum was absorbed mainly by the lymphatics in the dog when injected subcutaneously or intra-

peritoneally. Barnes and Trueta (1941) injected snake venom into the legs of rabbits after severing all the lymphatics and found that they survived whereas intact animals died. This suggests that the larger protein molecules are absorbed mainly or probably wholly by the lymphatics.

Courtice and his colleagues labelled plasma protein with the dye T1824, and found that when protein labelled in this way was introduced into the pleural or peritoneal cavities, it was all or nearly all absorbed by the lymphatics (Courtice and Simmonds, 1949; Courtice and Steinbeck, 1950, 1951). This label has been shown to be a very firm one (Rawson, 1938; Allen and Orahovats, 1951) and proteins labelled in this way behave as unlabelled proteins as far as the rate of absorption is concerned. The advantage of labelling the proteins is that homologous plasma proteins or the animal's own protein can be used This may be important when assessing the function of the lymphatic system in returning to the blood stream those proteins which have escaped through the capillary membrane. In these experiments, as well as those of Lewis and of Field and Drinker, a small percentage of the absorbed dye generally entered the blood stream by some undetectable route This could be by small subsidiary entrances into the venous system of lymphatics which had not been tied off or by diffusion directly into the blood capillaries. As it is impossible to be absolutely sure that there is not a small branch duct entering the vein in the neck or some other vein as described in Chapter 1, it is not possible to be sure whether or not a small amount of protein can or does pass back through the blood capillary membrane In any case this is at most only a small amount as shown in Fig 28

Protein does not readily *re-enter* the blood capillaries directly because the pressures are not favourable for such a backward filtration Diffusion *must* play a negligible part for even when high concentrations of protein are introduced into the peritoneal cavities, to give a high protein concentration gradient from tissue fluid to plasma, there is no appreciable abs[illegible] by the blood capillaries

[illegible] re-enter the [illegible] nphatic vessels. There is thus set up an extravascular [illegible] plasma proteins and the main function of the lymphatics is this return to the blood stream of the protein that has escaped from the blood vessels Should the lymphatics become blocked as in filariasis or by experimental injection of a sclerosing agent into the vessels, elephantiasis will result. Drinker, Field and Homans (1934) produced blockage of the lymphatics of the leg of the dog by injecting into the cannulated lymphatics, crystalline silica and quinine hydrochloride. After repeated injections the lymphatic drainage of the hind leg became permanently impassable. There resulted



28 —The amounts of dye absorbed by the right lymph duct, thoracic duct and the blood-stream after the introduction of dye-labelled plasma into the peritoneal cavity of the cat Results expressed as a percentage of the dye injected

(Redrawn from Courtice and Steinbeck, 1950)

Fig 29 —Elephantiasis in a dog caused by repeated intralymphatic injections of quinine hydrochloride and crystalline silica.

(From Drinker, Field and Homans, 1934, Fig 6, p 516)

a progressive accumulation of oedema fluid which contained protein up to 4-5 g. per cent, an overgrowth of connective tissue with dilatation and thickening of the lymphatic capillaries and an increased susceptibility to streptococcal infection, Fig. 29. These experiments clearly demonstrate the normal function of the lymphatic vessels in removing extravascular protein from the tissue spaces.

Permeability of the capillary wall to protein

If normal plasma proteins, labelled with a radio-active isotope or with T1824, are injected intravenously, they can be detected in the lymph of the thoracic duct within 10 to 15 minutes and in other lymphatic ducts somewhat later (Courtice, 1943; Cope and Moore, 1944; Wasserman and Mayerson, 1951; Forker, Chaikoff and Reinhardt, 1952; Korner, Morris and Courtice, 1954). In Fig. 30 are plotted the concentrations of dye in the plasma and in the liver, thoracic duct and cervical

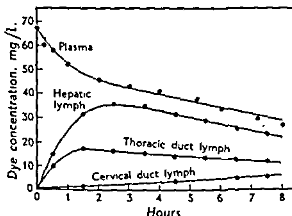


Fig 30.—The concentration of dye in the plasma and in the lymph from the hepatic, intestinal and cervical ducts after the intravenous injection of T1824 in the cat (By courtesy of Mr. Bede Morris)

duct lymph after the intravenous injection of T1824. These findings show quite clearly that protein is normally escaping from the circulating plasma into the extravascular fluid and returning to the blood stream by the lymphatics. Not only labelled homologous plasma proteins but also various foreign proteins have been shown to behave in this way (cf. Field and Drinker, 1931*a*; Lewis, 1921). In general, we shall see that the ease with which protein molecules pass through the capillary membrane depends primarily on the relative size of the molecules. The size and approximate shape of the normal plasma proteins are shown in Fig 31, and in Table 6 are given also the electric charges carried by the

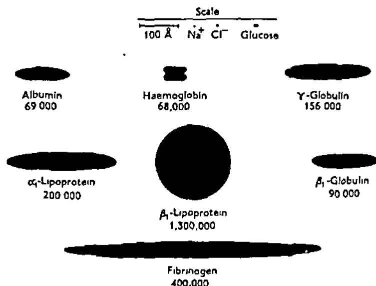


FIG 31.—The relative dimensions of various proteins
(From Janeway, 1949)

protein molecule. These figures show that most of the plasma protein molecules are of approximately the same equatorial diameter, but vary greatly in length.

TABLE 6

Dimensions and electric charge of plasma proteins and other plasma constituents

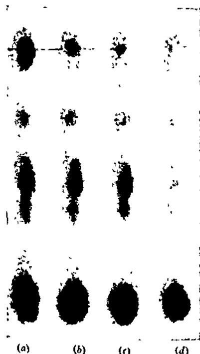
	Molecular weight	*Approx. dimensions* (Å) *Length*	*Equatorial diameter*	*Negative net charge per molecule at pH 7·4*	*Electric movement per molecule (Debye units)*
Sodium ion	23	1 9	1 9	− 1	—
Chloride ion	35 5	3 6	3 6	1	—
Glucose	180	9 5	6 5	0	—
Serum-albumin	69,000	150	38	18	400
Serum γ-globulin	156,000	320	36	8	1,200
Fibrinogen	500,000	900	33	—	—

From Cohn, Oncley, Strong, Hughes and Armstrong (1944)

Electrophoretic analysis of lymph reveals that all the plasma protein fractions escape from the blood capillaries. Using the Tiselius apparatus, Perlmann, Glenn and Kaufman (1943) found that lymph from the fore-legs of calves contains all the components observed in serum. The protein fractions have also been determined by paper electrophoresis. Courtice and Morris (1955) in this way examined lymph from the thoracic

and cervical ducts of dogs, thoracic, cervical and leg ducts of cats and of rabbits and thoracic duct of the rat. Typical results of these investigations are represented in Figs. 32 and 33.

Although all fractions of the plasma proteins are represented in the lymph, they are not present in the same proportions as in the plasma.



(a) (b) (c) (d)

Fig 32.—The electrophoretic patterns obtained by zone electrophoresis of (a) plasma, (b) hepatic, (c) intestinal and (d) cervical lymph of the cat. The horizontal line represents the point of application of the plasma or lymph

(By courtesy of Mr Bede Morris)

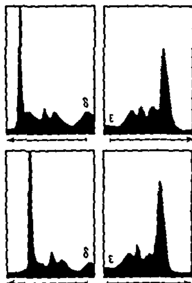


Fig 33.—The electrophoretic patterns of calf serum (upper diagram) and leg lymph (lower diagram) obtained by the Tiselius moving boundary apparatus

(From Perlmann, Glenn and Kaufman, 1943)

The albumin concentration is usually relatively greater than that of the globulin. Perlmann *et al.* determined the relative concentrations of albumin, α-, β- and γ-globulins in the serum and leg lymph of calves, Table 7. Whereas the mean albumin : globulin ratio in the serum was 1·28, that in the lymph was 1·69. This altered distribution of the proteins in lymph has also been shown by chemical analysis, Table 8. It is evident, therefore, that the smaller albumin molecules escape through the capillary wall somewhat more readily than the larger globulins. In a study of the exchange of labelled albumin and globulin between the

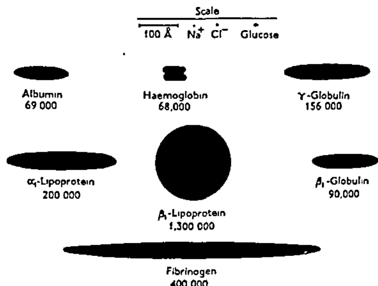


FIG 31 —The relative dimensions of various proteins
(From Janeway, 1949)

protein molecule. These figures show that most of the plasma protein molecules are of approximately the same equatorial diameter, but vary greatly in length

TABLE 6

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	Molecular weight	*Approx. dimensions* (Å) *Length*	*Equatorial diameter*	*Negative net charge per molecule at pH 7 4*	*Electric movement per molecule (Debye units)*
Sodium ion	23	1 9	1·9	− 1	—
Chloride ion	35 5	3 6	3 6	1	—
Glucose	180	9 5	6 5	0	—
Serum-albumin	69,000	150	38	18	400
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1932), gelatin with an equatorial diameter of 18 Å, and a molecular

rapidly as purified protein derivative from tuberculin with a molecular weight of only 14,500.

The behaviour of non-protein colloidal solutions also supports the view that molecular size in relation to pore size is the main factor determining the passage of these molecules through the capillary membrane. Wasserman and Mayerson (1952*b*) measured the concentration of dextran, with an average molecular weight of about 87,500, in the thoracic duct lymph of dogs after intravenous infusions. They found that the dextran appeared in the lymph in about 10 minutes and later reached a steady state between the plasma and lymph. The plasma-lymph gradient was larger than for albumin and globulin, which suggests that the dextran leaks from the plasma into the interstitial fluid more slowly than do the plasma proteins. Evidence was shown, however, that the passage of dextran through the capillary wall depended upon the molecular weight of the dextran. When, for example, dextran of molecular weight of 17,000 was used, it rapidly passed into the lymph and urine and the lymph concentration soon rose even above that of plasma, whereas when larger molecules were used, molecular weight 125,000, there was a wider plasma-lymph gradient indicating a much slower passage through the membrane. More recently Wasserman, Loeb and Mayerson (1955) have used eight well-characterized fractions of dextran of different molecular sizes, and their results indicate that the penetration of colloid molecules through capillaries is inversely related to molecular size, Fig 34 Similar results have been obtained with

pass are beyond the powers of any method of direct observation and measurement. Their magnitude may only be determined indirectly by

of 38 Å or possibly more.

More recently Pappenheimer and his colleagues have critically reviewed the pore theory of capillary permeability (Pappenheimer, Renkin and Borrero, 1951, Pappenheimer, 1953). They have concluded that uniform cylindrical pores of diameter 60 to 90 Å would account for the passage of lipid-insoluble molecules of various sizes through the capillary

TABLE 7

The electrophoretic distribution of proteins in leg lymph and serum of the calf. In the first three experiments samples of plasma and lymph were analysed in potassium phosphate buffer of *p*H 7·7 and in the last two in sodium diethylbarbiturate buffer of *p*H 8·6.

SERUM					LYMPH				
Total g. %	*Percentage of total*				*Total g. %*	*Percentage of total*			
	Albumin	*Globulins*				*Albumin*	*Globulins*		
6·04	55·8	11·4	12·7	20·1	2·87	67·6	5·1	11·4	15·9
6·75	61·0	9·8	8·9	20·3	3·34	63·2	9·2	10·5	17·1
5·44	55·8	13·2	14·8	16·2	2·47	63·1	9·4	12·4	15·1
6·30	53·7	19·4	17·5	9·4	2·46	63·0	14·8	15·1	8·1
6·19	55·1	16·0	16·5	12·4	2·90	58·0	16·6	15·4	10·0
6·14	56·3	14·0	14·1	15·7	2·81	63·0	11·0	13·0	13·2

From Perlmann, Glenn and Kaufman (1943)

TABLE 8

The albumin and globulin in serum and lymph.

	Protein g. %		*Albumin g. %*		*Globulin g. %*		*A/G ratio*	
	S.	*L.*	*S.*	*L.*	*S.*	*L.*	*S.*	*L.*
Thoracic duct								
Dogs	6·25	4·00	3·61	2·45	2·63	1·54	1·46	1·72
Cats	7·36	5·12	3·65	3·01	3·71	2·11	1·00	1·43
Cervical lymph								
Dogs	6·25	3·63	3·61	2·36	2·63	1·26	1·46	1·96
Cats	6·98	3·31	3·98	2·14	3·00	1·17	1·30	1·83
Leg lymph								
Dogs	6·46	1·91	3·62	1·20	2·84	0·71	1·38	1·81
Intestinal lymph								
Dogs	6·23	3·98	3·67	2·42	2·57	1·56	1·43	1·55
Heart lymph								
Dogs	5·95	3·83	2·98	2·20	2·96	1·64	1·00	1·39

Compiled from several sources

plasma and thoracic duct lymph, Wasserman and Mayerson (1952*c*) concluded that albumin leaks out of the capillaries approximately 1·6 *times faster* than globulin.

Evidence from the use of proteins of various size but not normally present in the plasma, in general support this view that smaller molecules can pass through the membrane more readily than the larger. Haemoglobin passes through the capillary more readily than albumin (Haynes,

degree of molecular sieving at normal rates of filtration" (Pappenheimer, 1953).

In this interpretation of the Pore Theory of capillary permeability, therefore, the different degrees of escape of the various proteins through the capillaries are explained by the concept of restricted diffusion through an isoporous membrane, the size of the pores being somewhat greater than the diameter of the largest molecules that pass. If however the pores are approximately the size mentioned above, this theory does not explain the escape of the large β-lipoprotein molecules (see p. 98) Our whole concept of the nature of the capillary barrier may, however, have to be modified in the future in the light of results obtained by electron microscopy (cf. Pease, 1955).

Gradient of permeability

In his original hypothesis, Starling assumed that the permeability of a capillary was the same throughout its length. Rous and his associates, however, gave evidence which indicated that certain dyes could pass through the venular end of the capillary more easily than through the arterial end (Rous, Gilding and Smith, 1930; Rous and Smith, 1931; Smith and Rous, 1931*a* and *b*, Smith and Dick, 1932; Hudack and McMaster, 1932). These authors believe that the gradient is an actual property of the vascular endothelium, not the result of such factors as changes in the blood or in blood pressure; and that it aids in maintaining a uniform environment for the tissues, since regions served by the venous part of the capillary will receive fresh filtrate, just as do those near arterial vessels. Chambers and Zweifach, in their experiments on capillary permeability and on capillary blood flow, have confirmed the observations of Rous and his associates. They conclude that there is a gradual increase in the relative number of larger pores toward the end where the blood is most venous. By re-routing the venous blood through a thoroughfare channel, they found that a new gradient is established with an increasing porosity toward the original arterial side of the capillary bed. These observations led them to suggest that the gradient appears to be a result of some factor in venous blood and not to permanent, structural differences in the vessel wall (Zweifach, 1940; Chambers and Zweifach, 1947)

Other possible factors in permeability of capillary membrane to protein

In the above views, then, the passage of various-sized molecules is considered to be determined merely by the relation of molecule to pore size. The question of other factors such as the electric charge carried by the molecule in relation to the charge of the pore has received less attention Webster, Engel, Lang and Amberson (1934–1935) perfused

membrane. It has been shown in artificial membranes that diffusion of molecules is restricted when the dimensions of the pores are comparable with those of the diffusing molecules. In such conditions the molecules will not diffuse as rapidly as their free diffusion coefficient would indicate This restricted diffusion can account for the degree of molecular sieving, i e. the ratio of the molecular concentration in the plasma to that in the filtrate, observed in filtration through the capillary membrane. Thus "according to this concept of restricted diffusion the effective pore size in the capillary walls is sufficiently great to allow even large plasma protein molecules to penetrate through the pores. The degree of molecular

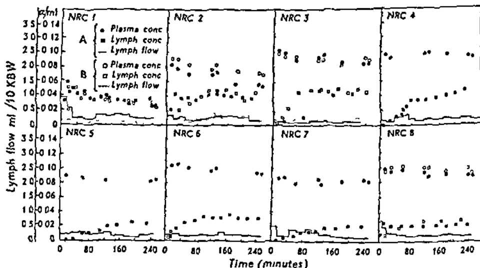


FIG 34—Plasma and lymph dextran concentrations for the eight N R C fractions in anaesthetized nephrectomized mongrel dogs plotted against time

The average molecular weights of the fractions were 10,600, 35,000, 51,300, 91,700, 135,000, 104,000, 255,000, 412 000 The concentrations are expressed in degrees of rotation (α/ml), the units of measure obtained from the polarographic method of analysis A and B represent two different dogs plotted on the same frame The ratio Plasma concentration/Lymph concentration is directly proportional to molecular size

(From Wasserman, Loeb and Mayerson, 1955)

sieving of any given solute depends upon the ratio of its restricted diffusion coefficient to the rate of volume flow through the capillary wall (filtration rate). For small molecules the restriction to diffusion is small, relative to physiological rates of filtration, and no appreciable concentration gradients are maintained across the capillary membranes. For large lipid-insoluble molecules the restriction to diffusion becomes so great that the degree of molecular sieving is determined largely by the rate of filtration. Thus molecular sieving of inulin through the capillary walls in muscle is readily demonstrable at high rates of filtration produced by venous congestion. In cases of still larger molecules, including the plasma proteins, the restriction to diffusion is sufficient to allow a high

the capillary filtrate than at the venular end or near those capillaries where reabsorption of fluid is occurring. As we have already stated, lymphatic capillaries may be draining an area near a filtering capillary or near a reabsorbing capillary and the protein content of the lymph in such vessels will no doubt vary. But, from moment to moment the process of filtration and reabsorption in blood capillaries is changing, which would tend to make the protein concentration in the tissue fluid more uniform in any region. Moreover, the lymph in the larger regional ducts, coming as it does from countless lymphatic capillaries from all positions relative to the blood capillaries, should be a fair average sample of the tissue fluid in the region drained by the duct. We can, therefore, conclude that the protein concentration of the lymph and of the tissue fluid in any area is approximately the same. This view was put forward by Drinker and his colleagues (Drinker and Field, 1931; Field and Drinker, 1931*a*; Drinker and Yoffey, 1941; Drinker, 1946) While they could not collect interstitial fluid from normal tissues and compare its composition with that of lymph, they showed that in oedema due to blockage of the lymphatics the protein content of the lymph and of the tissue fluid was approximately the same, Table 9 (Drinker, Field, Heim and Leigh, 1934).

TABLE 9

The protein content of lymph and oedema fluid from dogs 1, 2, 3 and 4 with experimental lymphoedema of the hind legs

	Date	*Leg*	*Protein in g per 100 ml.* *Lymph*	*Oedema fluid*
Dog 1 . .	Feb. 25, 1933	Left	2 67	2 00
	Mar. 8, 1933	Right	2 45	2 20
Dog 2	Sept 15, 1933	Left	3 37	3 37
	April 3, 1934	Left	2 48	3 45
Dog 3	Mar 28, 1933	Left	2 28	2 75
	May 5, 1933	Left	2 97	3 17
	Oct 16, 1933	Left	3 17	3 17
	April 6, 1934	Right	2 50	2 67
Dog 4	April 9, 1934	Left	2 55	1 86

Even though we accept this view, the variation in the protein concentration of lymph in different regions of the body prevents us from making a direct estimate of the extravascular protein pool The amount of extravascular protein which is exchangeable with the plasma proteins has, however, been determined indirectly by the isotope dilution principle. When labelled proteins are injected intravenously they reach an equilibrium with the extravascular protein pool during the first two or

the frog's kidney with a solution of haemoglobin over a range of *p*H from 5·5 to 7 8 and observed that at a low *p*H the haemoglobin passed through the capillary membrane more readily than at a high *p*H. A possible explanation of this was that at a low *p*H the haemoglobin would behave as a cation and pass the negatively charged glomerular membrane with greater ease, although it was realized that at these low *p*Hs the pore size of the membrane might be affected. Bott and Richards (1941) concluded from their experiments, in which they determined the concentration in the glomerular filtrate or in the urine of various injected proteins, that although the permeability of the membranes to protein follows approximately the size of the molecules, the sign and magnitude of the net charge may be of some importance. It is possible that the shape of the molecule and the position of the charge on it may enable some elongated molecules to be orientated so that they would more readily pass through the pores.

On the other hand dextran which electrophoretically behaves as an electrically inert substance (Kunkel and Tiselius, 1951) behaves like proteins in its passage through the capillary membrane. Molecules of 17,000 molecular weight rapidly escape from the circulation, those of 87,000 molecular weight behave approximately like albumin, while those of 125,000 molecular weight escape less readily than albumin (Wasserman and Mayerson, 1952*b*). This evidence suggests that molecular size in relation to pore size is the most important factor in the escape of the normal plasma proteins through the capillary membrane.

Extravascular Circulation of Plasma Protein

It is clear from the experiments that we have so far considered that the plasma proteins slowly escape through the capillary walls, mix with the proteins in the tissue fluid and ultimately enter the lymphatic capillaries to be returned to the blood stream In this circulation of the plasma proteins from the blood into the tissue fluid and back to the blood through the lymphatics, we may regard the plasma protein in the extravascular fluid as a "protein pool"

It is difficult to measure accurately the extent of this protein pool. The total volume of extravascular fluid can be determined, but tissue fluid cannot be collected for the estimation of the protein concentration. The only fluid that we can collect and examine is lymph ; and the relation between the protein concentrations of tissue fluid and of lymph has been controversial. In any tissue it seems likely that the level of protein in the interstitial fluid itself is not uniform. At the arteriolar end of a capillary or near any part of a capillary where filtration is occurring, the protein content of the fluid will more nearly resemble that of

tion of the tissue fluid and lymph will fall, without implying any change in the size of the capillary pores. It is, therefore, difficult to reach any definite conclusions concerning the size of the capillary pores in different tissues of the body, merely from measurements of the protein concentration in the lymph.

By measuring the rate of lymph flow as well, the total amount of protein leaking from the capillaries in a given region may be calculated. Without a knowledge of the area of the vascular bed and of the haemodynamics in the region, even this measurement cannot give an accurate estimate of a change in the pore size, unless the leakage of protein is grossly increased as in tissue injury. The total amount of protein escaping from the blood stream in any region, however, does serve as a guide to the physiological significance of the lymphatics in returning extravascular protein to the blood. Most figures available for the flow of lymph from various regions of the body are from anaesthetized animals, in whom lymph from the limbs or from the cervical ducts has been obtained by massage or passive movement. In Table 10 are collected

TABLE 10

The average flow and protein content of lymph from various lymph ducts in dogs under nembutal anaesthesia. The figures in parentheses represent the number of animals in each group. The average weight of each animal in each group was approximately 21 kg. and the average intravascular plasma protein 55 g. The total lymph protein from the leg and cervical ducts includes both sides

	Lymph flow	*Lymph protein*		
	ml /hr.	*g %*	*g /day*	*% of total intravascular plasma protein*
Thoracic duct (4)	26·0	4·1	26·1	47·5
Right lymph duct (21)	2·3	3·7	2·0	3·6
Cervical ducts (18)	2·2	2·8	1·3	2·4
Fore-leg ducts (5)	3·8	1·3	1·2	2·2
Hind-leg ducts (17)	2·2	1·8	1·0	1·8

From Courtice (1951)

data from a series of dogs under nembutal anaesthesia. Although these figures cannot be an accurate measure of the protein leakage in a normal, unanaesthetized animal, they show that by far the greater part of the escape of protein occurs in the liver and alimentary tract. These observations that plasma proteins more readily escape from the capillaries of the liver and alimentary tract than from the thoracic viscera, from the tissues of the head and neck, or from the limbs, are borne out by the findings that labelled plasma protein injected intravenously soon appears in the thoracic duct lymph, but only slowly in lymph from other regions of the body.

three days, when it is found that about half the injected protein remains in the circulation. It seems, therefore, that in man and animals the total extravascular protein is of the same order of magnitude as the intravascular (Sterling, 1951, Myant, 1952; Wasserman and Mayerson, 1951, Gitlin and Janeway, 1953; Fink *et al.*, 1944). While the total extravascular plasma protein of the body can be estimated in this way, it is not possible to calculate with any degree of certainty the magnitude of the extravascular pool in a given region. However, in a steady state the pool presumably remains constant; in these circumstances, the rate of protein removal in the lymph should equal the rate of protein leakage from the capillaries. This would not apply of course when the extent of the protein pool is rapidly changing as the result of some acute experimental interference.

Protein leakage in different tissues

It has just been suggested that the protein *output* in the lymph under certain conditions reflects the protein leakage from the capillaries However, it should be emphasized that the *concentration* of protein in the lymph is not by itself a measure of the permeability of the capillary wall. In Chapter 4, detailed figures of the protein composition of lymph from various tissues of the body are tabulated. The fact that lymph from all regions contains all the plasma protein fractions, and a higher A : G ratio than plasma, probably means that there is not any great difference in the size of the pores in the various capillaries. Nevertheless, it is generally believed that protein can more readily escape from some capillaries than from others If we take from this table (Table 32, Chapter 4) an average of the figures quoted for the dog, the animal for which most data are available, we find that expressed as a percentage of the protein concentration of the plasma, the protein levels in the lymph are as follows: Liver, 81; right lymph duct, 69; heart, 65; thoracic duct, 60; intestines, 54; cervical duct, 48 and skin, 28.

The fact that the protein concentration in the lymph from the lungs is 69 per cent, whereas that in the lymph from the intestines is only 54 per cent of the plasma protein level, does not mean that the lung capillaries are more permeable to protein than the intestinal capillaries. As Starling has made clear, in a steady state the protein concentration in the tissue fluid will be such that the effective osmotic pressure exerted by the plasma proteins will balance the capillary pressure (see p. 65). Other things being equal, therefore, the protein concentration in the interstitial fluid of a tissue such as the lungs, where the capillary pressure is low, will be higher than that in the intestines where the capillary pressure is much higher. Again, in any one tissue, if the filtration rate is increased by increasing the capillary pressure, the protein concentra-

metabolism by the use of radio-active isotopes. They have shown clearly the dynamic state of the body constituents (Schoenheimer, 1941). With regard to protein their experiments have shown the rate of interaction of N^{15}-labelled amino acids with body proteins. In particular they have measured the turnover of protein or the protein activity in various tissues Of all the tissue proteins, they have found that the serum proteins are *the most active, followed closely by the proteins* of the liver and intestines, whereas the protein activity of the skin and the muscles is much less (Shemin and Rittenberg, 1944; Sprinson and Rittenberg, 1949).

The results of these experiments and of others (cf. Abdou and Tarver, 1951*a* and *b*) are suggestive when taken with determinations of extravascular protein turnover. By far the greatest extravascular circulation of protein occurs in the abdominal viscera. Perhaps this may be related to the greater turnover or metabolism of protein in these organs. In the normal animal, therefore, we find not only that the plasma proteins are essential in maintaining the fluid balance across the capillary membrane, but also that they are essential in the normal protein metabolism of the cell, and whether it be merely coincidence or not we observe that the protein leakage through the capillary membrane is greatest in those tissues which have a high protein metabolism or a high rate of protein turnover.

Factors affecting the Extravascular Protein Pool and the Extravascular Circulation of Protein

Capillary pressure

Since the total protein content of the lymph coming from any region is a measure of the protein escaping from the blood stream in that region only when the extravascular pool of protein of that tissue remains constant, it is often difficult to interpret, in terms of capillary filtration, changes in the flow and composition of lymph which are of short duration

When a labelled protein is injected intravenously, the specific activity (i.e. concentration of labelled protein/total protein concentration) of the lymph gradually rises until it reaches that of the plasma in 7 to 8 hours in the case of the thoracic duct (cf. Cope and Moore, 1944; Wasserman and Mayerson, 1952; Korner, Morris and Courtice, 1954). This shows that the labelled protein escaping from the capillaries mixes with the unlabelled protein in the extravascular pool. The lymph which is draining away the tissue fluid contains gradually increasing amounts of labelled protein, i e. protein which has escaped from the blood stream. The rate of increase of specific activity of the lymph is a measure of the rate of turnover of the protein in the extravascular pool and will depend on the size of the pool and the rate of protein leakage through the capillaries

Total extravascular circulation of protein

Since the thoracic duct returns such a large proportion of the protein that becomes extravascular each day, this amount of protein has been taken as a guide to the total extravascular circulation. In the experiments on dogs quoted in Table 10, about 50 per cent of the intravascular plasma protein escaped into the tissues per day. Other workers have reported similar amounts. Forker, Chaikoff and Reinhardt (1952) estimated that in unanaesthetized rats about two-thirds and in anaesthetized dogs about half the plasma protein was lost from the blood stream per day, while Courtice and Morris (1955) found that the thoracic duct lymph contained protein in average amounts equal to 84 per cent of the circulating plasma proteins in cats, 62 per cent in rabbits and 57 per cent in dogs. If we allow for the fact that some extravascular protein is absorbed by the cervical, foreleg and right lymph ducts, it would be reasonable to assume that from 50 to 100 per cent of the plasma proteins pass through the capillaries into the extravascular compartment each day.

Significance of the extravascular circulation of protein

We should briefly consider the significance of this relatively considerable extravascular circulation of plasma protein. It is obvious that circulating antibodies, for example, must escape from the blood stream to be completely effective. Some smaller molecules such as iron and cholesterol are transported into the tissue fluid bound with protein. In recent years, however, much attention has been focused on the part played by the plasma proteins in nitrogen metabolism.

A great deal of evidence has now accumulated which suggests that the plasma proteins play a very active part in the protein metabolism of the body. Whipple and his colleagues, in a long series of papers on plasma proteins and protein metabolism in general, have shown that animals can be kept in nitrogen equilibrium with intravenous injections of plasma protein as the only source of nitrogen, and that in the metabolism of these proteins by the cells the molecules need not be first broken down to amino acids. They maintain that the amino acids from ingested protein are first formed into plasma proteins in the liver and the exchange with cells takes place between the proteins of the cells and the plasma proteins (Daft, Robscheit-Robins and Whipple, 1938; Howland and Hawkins, 1938, Madden and Whipple, 1940; Whipple, 1942; Yuile, Lamson, Miller and Whipple, 1951). For this to be true, the extravascular circulation of plasma protein must be an essential part of the protein metabolism, for it is only by the escape of the proteins from the circulation that they can come into contact with tissue cells.

Schoenheimer and his colleagues have studied many aspects of protein

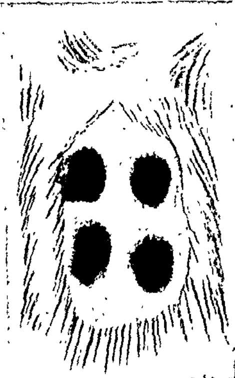


FIG 35 —Increased permeability of the vessels of the skin of the rabbit's abdomen produced by injections of broth

The blue spots are due to the leakage of dye-protein after the intravenous injection of Trypan blue or T1824 (From Menkin, *Dynamics of Inflammation*, Macmillan, 1940)

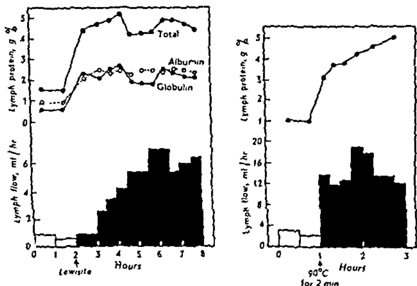


FIG 36 —The flow and protein content of the lymph from a limb after the local application of a vesicant, lewisite, and after a thermal burn

(Redrawn from Cameron, Courtice and Short, 1947, and Courtice, 1946)

Whenever the capillary pressure is raised in any region, the turnover of extravascular protein is increased. Transfusion of Ringer-Locke solution, for example, speeds up the rate of filtration both of fluid and of protein. If, under such circumstances, labelled protein is injected intravenously, its escape from the blood stream and mixing with the protein pool are speeded up, so that the time taken for the specific activity of the lymph to reach that of the plasma is decreased (Wasserman and Mayerson, 1952*a*; Korner, Morris and Courtice, 1954). With very large transfusion rates, the turnover is so great that the composition of lymph probably approximates that of the capillary filtrate. Wasserman, Loeb and Mayerson (1955) give evidence which suggests that the pores in the capillary wall stretch when the blood volume is significantly increased

Damage to endothelium

Another cause of increased escape of protein from the blood stream is injury. Labelled protein injected intravenously into an animal which has an injury will rapidly accumulate in the tissue fluid of the damaged tissues. This can readily be observed if either of the blue dyes T1824 or Trypan blue, which combine with plasma albumin, is injected intravenously as a label for the plasma protein. Any injured tissue will rapidly become deep blue, Fig. 35 (cf. Menkin, 1940).

In such cases the flow and protein content of the lymph from the injured area are greatly increased (Fig. 36). The specific activity of the lymph very rapidly approaches that of the plasma (Cope and Moore, 1944). There can be no doubt that in these circumstances the permeability of the capillary membrane to protein is greatly increased, since the greatly increased escape of protein through the capillaries cannot be explained merely by an increase in capillary pressure. The function of the lymphatic vessels in injury will be discussed in more detail in Chapter 8.

Oxygen deficiency

While there is general agreement that infusion of fluids increases filtration mainly by raising the capillary pressure, and injury does so by altering the permeability of the capillary membrane, there is still disagreement regarding the effects of acute oxygen deficiency on capillary filtration. With extreme grades of hypoxia or complete anoxia, Landis (1927–1928) demonstrated an increased permeability of the capillaries of the frog's mesentery. More recently, Henry, Goodman and Meehan (1947) and Hendley and Schiller (1954) also found that very severe hypoxaemia increased the permeability of the capillaries in the perfused isolated hind legs of rats.

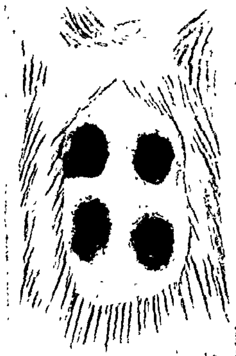


Fig 35 —Increased permeability of the vessels of the skin of the rabbit's abdomen produced by injections of broth

The blue spots are due to the leakage of dye-protein after the intravenous injection of Trypan blue or T1824 (From Menkin, *Dynamics of Inflammation*, Macmillan, 1940)

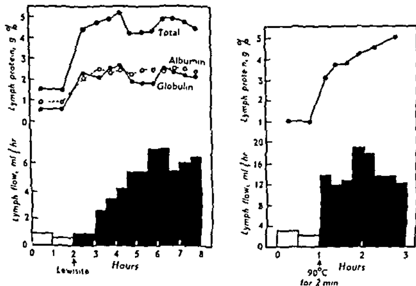


Fig 36 —The flow and protein content of the lymph from a limb after the local application of a vesicant, lewisite, and after a thermal burn.

(Redrawn from Cameron, Courtice and Short, 1947, and Courtice, 1946)

The effect of more physiological degrees of oxygen deficiency, however, is still debatable. Drinker and his colleagues have put forward the concept that capillary permeability is a physiological variable that is related to the oxygen tension in the tissues. They base this concept on experiments in which they studied the lymph flow from different regions under varying degrees of oxygen deficiency. Maurer (1940–1941; 1941) investigated the effects on the cervical and cardiac lymph flow and lymph proteins of hypoxaemia produced by the inhalation of air deficient in oxygen and of air containing carbon monoxide. He found

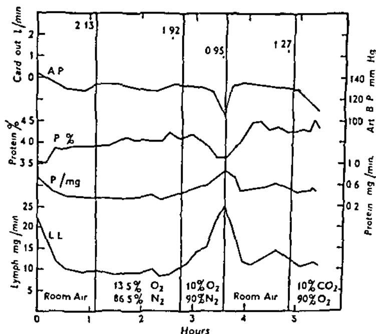


FIG 37.—The effects of oxygen lack on flow of lung lymph.

LL, lung lymph, P mg, protein in lymph, mg/min, P %, protein per cent in lymph, AP, arterial pressure in mm mercury

(From Warren, Peterson and Drinker, 1942)

that in all cases hypoxaemia, however produced, caused a sharp rise in lymph flow and a fall in the protein concentration. This rise in lymph flow, however, did not persist for long even though the hypoxaemia persisted, and it rapidly returned to normal when air or oxygen was breathed. Similar results have been obtained by investigations of the

Warren, Peterson

of 10 per cent

flow and a fall

in the concentration of the lymph proteins, Fig 37. With the thoracic duct lymph, Beznak and Liljestrand (1949) obtained similar results.

These changes in lymph flow have been interpreted as being due to an increased permeability of the capillaries caused by the lowered oxygen tension. There seems no doubt that the increased lymph flow in these experiments is the result of increased capillary filtration. It is difficult, however, to ascertain whether this is caused by increased permeability of the capillary membrane or by an increased capillary pressure. An increased lymph flow with a decreased protein concentration of the lymph may result from altered haemodynamics, such as occurs in a limb when it is warmed in a water-bath. If this interpretation were applied to these experiments, the rapid reversibility of the postulated increase in capillary permeability when the hypoxaemia was abolished could then be explained by a return to normal haemodynamics. In Fig. 37, the rapid rise in lymph flow occurred at a time when the cardiac output fell to half the normal level, so that the haemodynamics of the pulmonary circulation were undoubtedly altered

Courtice and Korner (1952) showed quite clearly that pulmonary oedema was more readily produced in rabbits transfused with Ringer-Locke solution when they were breathing 10–11 per cent oxygen than when they were breathing air. They gave some evidence to suggest that this was due to a raised pressure in the pulmonary vessels due to a lowered output of the left ventricle. A similar effect on the fluid balance in the lung was obtained by *l*-noradrenaline, which causes a vasoconstriction of the small blood vessels of the systemic circulation with a consequent shunting of the blood to the low pressure systems, the veins and the pulmonary circulation (Korner, 1953). These effects of systemic vasoconstriction on the haemodynamics of the pulmonary vascular bed have been clearly demonstrated by Sarnoff and his colleagues (Sarnoff and Sarnoff, 1952, Sarnoff, Berglund and Sarnoff, 1953, Sarnoff and Berglund, 1952) Although these experiments do not prove that there is no alteration of capillary permeability in moderate hypoxaemia, they do show that increased filtration due to increased capillary pressure resulting from a redistribution of the blood must also be considered. While very severe hypoxaemia may actually increase the permeability of the lung capillaries to protein, it seems that with moderate degrees of oxygen deficiency any increase in protein leakage may be mainly attributed to increased capillary pressure, without postulating alteration in permeability of the capillary membrane

Other experiments support the view that moderate hypoxaemia does [illegible] of the capillary mem- [illegible] Warren, 1944, Henry, [illegible] rtice, 1954, Hendley and Schiller, 1954). Using the perfused, isolated hind leg of the rat, Hendley and Schiller found that with moderate hypoxaemia, 5 to 10

volumes per cent of oxygen, little or no increased filtration occurred, but when the oxygen in the perfusing fluid was reduced to 0 to 2 volumes per cent, filtration increased considerably. They came to the conclusion from these experiments that hypoxaemia over a wide physiological range does not alter capillary permeability, but that at a critical oxygen content an abrupt increase in capillary permeability occurs.

These experiments on the effect of oxygen deficiency on capillary filtration emphasize what has earlier been said concerning the difficulties in interpreting changes in capillary filtration. Whereas gross changes in protein leakage through the capillaries undoubtedly signify an alteration in permeability, smaller changes can only be interpreted as such when we have an accurate estimate of the blood flow and pressure in these vessels

The Passage of Lipids through the Capillary Membrane

We have seen how the Pore Theory of capillary permeability explains the exchange of small ions and molecules and of proteins through the capillary membrane. In recent years much attention has been focused also on the transport of lipids—the physical state in which they are carried in the blood and the way in which they are exchanged between the circulating blood and the cells. The plasma lipids—neutral fat, fatty acid, phospholipid and cholesterol—are highly water-insoluble substances in themselves, yet they are present in the plasma of fasting animals in a state of optical clarity. How these lipids cross the endothelial membrane will depend to a large degree on their physical state in the plasma.

Physical state of plasma lipids

In any one individual in the postabsorptive state, the concentrations of plasma lipids are relatively constant from day to day, but these values may vary between individuals of the same species and between species of different dietary habits (cf. Bloor, 1943). In Table 11 are given figures for several species. It is clear that in man and dog the cholesterol level is much higher than in the herbivora, with the exception of the horse. Some of the fat is in the form of finely emulsified droplets or chylomicrons (Gage and Fish, 1924, Frazer and Stewart, 1937). The exact composition of these particles, which vary in size up to 1 μ in diameter, is not known. They consist very largely of neutral fat; in addition they contain some cholesterol (Swank and Wilmot, 1951; Necheles, 1952) and are probably stabilized by a film of adsorbed protein and phospholipid (Laurell, 1954). Robinson (1955) found that lipid particles prepared from rat chyle were stabilized by a film of phospho-

TABLE 11

The lipid concentrations in the plasma of various animal species.

		Total fatty acid mEq/l	*Total cholesterol* mg. %	*Phospholipid phosphorus* mg %
Human	(10)	12·1 ± 0·6	213 ± 19·4	10·97 ± 0·5
Dog	(6)	12·2 ± 0·9	194 ± 35·0	13·00 ± 1·4
Cat	(20)	10·8 ± 0·9	98 ± 7·3	7·40 ± 0·3
Rat	(5)	10·4 ± 2·6	43 ± 6·6	5·50 ± 0·3
Mouse	(6)	10·0 ± 0·4	97 ± 4·4	6·97 ± 0·6
Ox	(4)	4·0 ± 0·5	63 ± 9·0	5·02 ± 1·0
Sheep	(4)	6·1 ± 0·8	64 ± 12·0	5·23 ± 1·3
Goat	(1)	4·6	34	3·26
Horse	(4)	8·7 ± 0·4	128 ± 12·0	7·66 ± 0·4
Rabbit	(10)	11·4 ± 0·6	46 ± 8·8	4·20 ± 0·7
Guinea-pig	(6)	5·3 ± 0·6	50 ± 3·6	2·70 ± 0·2

The mean results are given together with their standard errors. The figures in parentheses indicate the number of animals in each group.

From Morris and Courtice (1955*a*)

lipid only and contained no protein. During fat absorption from the alimentary tract the chylomicron count in the plasma increases considerably, so that during alimentary lipaemia the increase in plasma lipids is largely in this form. The major part of the plasma lipids in the postabsorptive animal, however, is more intimately combined with the plasma proteins as true lipo-protein molecules. The existence of these protein-lipid complexes was demonstrated by Macheboeuf in 1929 when he isolated a protein from horse serum with a lipid content of some 40 per cent. Since this time the properties of the plasma lipoproteins have been extensively studied.

In fasting human plasma the lipids, apart from some chylomicrons, exist almost exclusively in the form of two distinct types of lipoprotein, having electrophoretic mobilities of the alpha and beta globulins. The alpha lipoprotein with a molecular weight of 200,000 and molecular dimensions of approximately 50 Å × 300 Å represents about 3 per cent of the total plasma proteins and contains about 35 per cent lipid; the beta lipoprotein with a molecular weight of 1,300,000 and molecular dimensions of approximately 185 Å × 185 Å represents about 5 per cent of the total plasma proteins and contains about 75 per cent lipid. The major part of the plasma cholesterol and phospholipid is contained in these lipoproteins, both of which contain phospholipids, unesterified cholesterol and cholesterol esterified with fatty acids. The ratio of cholesterol to phospholipid is higher in the beta than in the alpha fraction (cf. Oncley, Scatchard and Brown, 1947; Gurd *et al.*, 1949; Oncley and Gurd, 1953). While human plasma has a large complement of beta lipoprotein, most animals have a preponderance of lipoprotein migrating

electrophoretically in the alpha globulin-albumin area, Fig 38 (cf. Morris and Courtice, 1955*a*) In the herbivora with low plasma cholesterol concentrations, the lipoproteins are not so pronounced as in the dog and cat.

Gofman and his associates have used a different technique for separating the lipid-protein complexes of plasma, the analytical ultracentrifuge. In a medium of greater density than their specific gravity, the lipid complexes undergo flotation, the flotation rates being proportional to

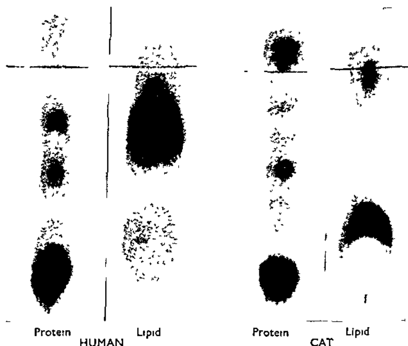


FIG 38—The electrophoretic patterns for human and cat plasma stained for protein (bromophenol blue) and for lipid (Sudan black)
The cross lines represent the points of application of the plasma on the filter paper
(From Morris and Courtice, 1955*a*)

their size and specific gravity In this way the lipoprotein associations have been characterized by their specific flotation rates into at least four groups, (i) the species which migrate with –Sf values greater than 75 units. These include the chylomicrons and aggregates of much smaller dimensions. (ii) The species which migrate with –Sf values between 30 and 70 units (iii) Those with –Sf values 10–20 which have a molecular weight of about 3,000,000. (iv) Those with –Sf values 3–8. These represent the beta lipoprotein (cf. Lindgren, Elliott and Gofman, 1951; Gofman *et al.*, 1950*a* and *b*).

As well as globulin-lipid associations, there are lipid-albumin com-

plexes. Plasma albumin possesses a considerable affinity for many different types of substances among which are the fatty acid anions. Kendall (1941) described a serum albumin which contained about 2 per cent fatty acid. Robinson and French (1953) and Robinson, Jeffries and French (1954) described an association between albumin and fatty acid in the plasma of rats following the injection of heparin. Morris and Courtice (1955*a*) have demonstrated by zone electrophoresis the presence of lipid in the albumin zone in samples of plasma in the rat and mouse In mouse plasma, in particular, a constant feature is a lipoprotein migrating at a faster rate than the main albumin fraction.

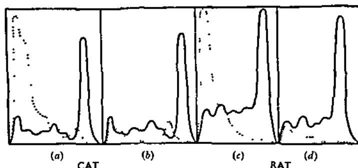


FIG 39 —Electrophoretic patterns shown as elution diagrams of the thoracic duct lymph from the cat and rat after a fat meal

(*a*) and (*c*), patterns of the milky lymph, (*b*) and (*d*), patterns of the lymph after the chylomicron layer had been removed by centrifugation at 20,000 g for 1 hour

——— Protein
.... Lipid

(From Courtice and Morris, 1955)

Ingested fat is absorbed into the blood stream by way of the intestinal lymphatics mainly in the form of chylomicrons. If fatty chyle or plasma is centrifuged at 20,000 g for an hour, the upper chylomicron layer may be removed, and the lipoprotein pattern of the lower layer remains the same as in the fasting state, Fig. 39 (Morris and Courtice, 1955*b*)

It is clear from this brief survey that the passage of the plasma lipids through the capillary membrane will depend not so much on the physical properties of the lipids themselves, but on those of the lipid-protein complexes The biological implications of these complexes is evident, since highly water-insoluble lipids are rendered water soluble by their association with hydrophilic colloids.

Permeability of capillary membrane to lipids

To investigate the passage of these lipids through the capillary membrane, the levels in the plasma and lymph have been measured in

the postabsorptive state when the chylomicrons are relatively few, and also after the intravenous injection of fatty chyle or artificial fat emulsions as well as during the normal digestion of fat when the thoracic duct is emptying chyle rich in chylomicrons into the blood stream.

In the postabsorptive state, the amount of exogenous lipid entering the thoracic duct from the alimentary tract is minimal, and in these circumstances the concentration of lipids is higher in the plasma than in the lymph. In lymph from the cervical duct the lipids are still less than in thoracic duct lymph and in general the lipid gradient from plasma to lymph runs roughly parallel with that of the plasma proteins, Table 12.

TABLE 12

The protein and lipid content of plasma, thoracic duct and cervical duct lymph in groups of cats and dogs in the postabsorptive state. Mean figures are given together with their standard errors

	Plasma	*Thoracic duct lymph*	*Cervical duct lymph*
Cat (20)			
Total protein g %	7·09 ± 0·14	4·63 ± 0·19	3·02 ± 0·28
Albumin g %	3·65 ± 0·14	2·74 ± 0·12	1·97 ± 0·22
Globulin g %	3·44 ± 0·05	1·88 ± 0·15	1·05 ± 0·07
Total fatty acid mEq/l	10·8 ± 0·9	9·1 ± 1·5	5·2 ± 0·9
Total cholesterol mg %	98·4 ± 7·3	43·6 ± 3·3	35·0 ± 6·4
Phospholipid mg %	193 ± 7·5	100 ± 7·5	97·5 ± 20·0
Dog (4)			
Total protein g %	5·65 ± 0·26	3·44 ± 0·42	2·57 ± 0·36
Albumin g %	3·67 ± 0·23	2·38 ± 0·23	1·72 ± 0·39
Globulin g %	1·97 ± 0·26	1·08 ± 0·21	0·85 ± 0·10
Total fatty acid mEq/l	14·0 ± 0·2	13·1 ± 1·9	5·3 ± 0·7
Total cholesterol mg %	258 ± 29	124 ± 7·9	67 ± 8·4
Phospholipid mg %	400 ± 10·0	223 ± 15·0	103 ± 5·0

By courtesy of Mr Bede Morris

All the different lipid-protein associations present in the plasma have been identified in the lymph (Page, Lewis and Plahl, 1953; Courtice and Morris, 1955). Using the technique of zone electrophoresis and staining the lipids with Sudan black, Courtice and Morris have identified the plasma lipoproteins in the lymph from the thoracic, cervical and leg ducts of the cat and rabbit, from the thoracic and cervical ducts of the dog and from the thoracic duct of the rat, Fig 40. In the cat's hepatic lymph, which contains nearly the same amount of protein as plasma, the lipoprotein concentrations are very high (Morris, 1956*a*) whereas in lymph from other tissues the levels are lower than in plasma. In all these animals, the alpha-lipoprotein predominates. When rabbits are fed cholesterol, however, the beta-lipoprotein fraction in the plasma may increase enormously with a much smaller rise in the alpha-lipoprotein.

In these circumstances the lymph contains beta-lipoprotein, but the evidence suggests that this leaves the circulation less readily than the alpha fraction (*Morris and Courtice, 1955b*). *In man who normally has a* preponderance of beta lipoprotein, no information is available concerning the presence of these lipids in lymph, but the results with animals

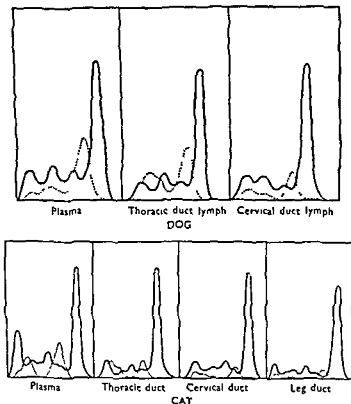


FIG 40—Elution diagrams of electrophoretic patterns of plasma and lymph from the dog and cat

(From Courtice and Morris, 1955)

suggest that the beta lipoprotein molecule will pass through the capillary membrane to a lesser extent than the smaller alpha fraction.

It is apparent, therefore, that just as the proteins of the plasma undergo an extravascular space-lymphatic circulation, so do the plasma lipids associated with protein as lipoproteins The lymph acts as a vehicle of transfer back to the blood stream for those lipids not utilized by the cells. We can probably explain the leakage of these lipoproteins through

the capillary membrane by the Pore Theory. If, however, in these animals the dimensions of the lipoprotein molecules are the same as those attributed to the lipoproteins of man, and if the pores are the size we have mentioned earlier in this chapter, the larger beta molecule should have difficulty in escaping, since its diameter is at least twice that of the pores.

Not only are there alpha and beta lipoproteins in the lymph in the postabsorptive state, but lymph from the cervical, hepatic and leg ducts—all draining tissues remote from the alimentary tract—also contains chylomicrons, although macroscopically it appears clear (Courtice and Morris, 1955; Morris and Courtice, 1956). We can readily understand how the intestinal lymph always contains chylomicrons, even in what we call the postabsorptive state. The presence of chylomicrons in the lymph from other tissues, however, suggests that they come either from the blood stream by passing through the capillary membrane or from the fat depots. The evidence indicates that the chylomicron count in the lymph may vary with that in the blood, which suggests that these particles may pass through the capillary membrane and so appear in the lymph. For example, the hepatic and cervical lymph ducts were cannulated in a fat-fed cat and chylomicron counts made on the lymph and on the plasma. The thoracic duct which was pouring very fatty chyle into the blood stream was then cannulated and the lymph collected. The chylomicron count in the plasma fell in the next few hours and with this fall the counts in the hepatic and cervical lymph also fell. The fatty chyle which had meantime been collected from the thoracic duct was then injected intravenously making the plasma quite milky. The chylomicron counts in the hepatic and cervical lymph subsequently rose. These changes are shown in Table 13.

When fatty chyle or an artificial fat emulsion is injected into the blood stream in this way, it very rapidly leaves the circulation. Although the chylomicron counts in the hepatic and cervical lymph rise, the actual increase in lymph lipids compared with the amount leaving the circulation is very small (Marble *et al*, 1934; Meng, 1952; Morris, 1954). Morris and Courtice (1956) have injected fatty chyle collected from fat-fed cats into postabsorptive cats, and determined the actual amount of lipid leaving the circulation and the amount appearing in hepatic, intestinal and cervical lymph. In Table 14 are the results of a typical experiment. In this experiment 60 ml. of fatty chyle containing 113.7 mEq/l. of total fatty acid (which represents 1,835 mg. of fat in all, expressed as tripalmitin) was injected and the plasma total fatty acid concentration rose from 4·3 to 29.0 mEq/l., but fell almost to its original level in an hour. The total fatty acid concentration of the hepatic lymph rose, but the total amount of lipid in all the hepatic lymph collected during the

TABLE 13

Chylomicron counts, average number in a given fixed area, in the plasma, hepatic and cervical duct lymph, in a cat fed previously on a diet rich in fat. After initial observations over a period of 3 hours, the thoracic duct was cannulated and the fatty chyle collected, four hours later the fatty chyle which had been collected was injected intravenously.

	Plasma 1 in 200 *dilution*	*Hepatic lymph* 1 in 200 *dilution*	*Cervical duct lymph*
Before cannulating thoracic duct	25	15	23
	—	20	—
After cannulating thoracic duct			
1 hour	—	18	—
2 "	—	10	22
3 "	—	9	13
4 "	14	8	12
20 ml. fatty chyle injected			
1 hour	—	12	11
2 "	—	14	20
3 "	—	18	36

From Morris and Courtice (1956)

TABLE 14

The effect of the intravenous injection of 60 ml. fatty chyle from a fat-fed cat, containing 113·7 mEq/l total fatty acid, into a fasting cat of 2·6 kg. weight.

		Before injection	*Immediately after*	*1st hr.*	*2nd hr.*	*3rd hr*
Plasma	Total fatty acid mEq/l.	4·3	29·0	7·5	6·9	6·7
	Chylomicron count	24	—	—	—	—
Hepatic lymph	ml./hr	1·3	—	1·2	1·2	1·4
	Total fatty acid mEq/l	3·9			5·8	
	Chylomicron count	16	—	34	30	15
Cervical lymph	ml./hr	0·2	—	0·2	0·3	0·2
	Chylomicron count	14	—	70	35	22
Intestinal lymph	ml./hr	2·2	—	2·8	2·6	2·1
	Total fatty acid mEq/l	6·7	—	5·7	2·4	3·6

From Morris and Courtice (1956)

3 hours after infusion was only one per cent of the amount injected. The actual increase in the lymph lipids over the pre-infusion level was only a third of this. The increase in lipid during the first hour from the intestinal duct was insignificant, while the flow from the cervical duct was so small that only a very small fraction of the escaping lipid appeared in this lymph. In this particular experiment, therefore, about

1,835 mg. of fat, introduced into the blood stream mainly as chylomicrons, passed through the capillary membrane within an hour and over 99 per cent of it was deposited and did not return in the lymph as do the lipoprotein molecules. The amount of protein leaving the circulation per hour was 77 mg. in the liver, 142 mg in the intestines and 11 mg. in the tissues drained by the cervical duct, that is only 230 mg per hour. In another cat 2,540 mg. of injected fat left the circulation within two hours during which time the leakage of protein was 448 mg. As we saw earlier in this chapter the amount of plasma protein leaving the circulation in regions of the body other than those drained by the hepatic and intestinal ducts is small It is clear, therefore, that fat in chylomicron form can disappear from the blood stream much more rapidly than protein.

The results of these experiments are in conformity with much evidence on the leakage of protein from the circulation, which we have discussed earlier in this chapter, and the rapid disappearance of fat from the blood stream after a fatty meal or after the intravenous injection of chyle or artificial fat emulsions (cf. Little, Harrison and Blalock, 1942; Meng and Freeman, 1948; Berry and Ivy, 1948; Lerner, Chaikoff and Entenman, 1949, Meng, 1952; Waddell *et al.* 1953) Many experimental investigations have been undertaken recently to ascertain the way in which chylomicrons, which are quite large particles compared with *alpha and beta-lipoprotein molecules, pass through the capillary membrane.* There is some evidence that the plasma chylomicrons may first be converted to the smaller lipoprotein molecules before leaving the circulation. Heparin, for example, injected intravenously, will clear lipaemic plasma following fat ingestion (Hahn, 1943; Swank and Wilmot, 1951) and leads to a redistribution of lipid between the plasma proteins (Nikkila, 1952; Fasoli, 1953, Herbst and Hurley, 1954) During the *in vitro* clearing of chyle by heparinized plasma, Robinson and French (1953) have shown that fatty acid is released and becomes associated with plasma albumin as a soluble fatty acid-albumin complex. The work of Robinson, Jeffries and French (1954) suggests that a similar process may be responsible for the physiological dissolution of chylomicrons *in vivo*

If, however, all absorbed neutral fat has first to be incorporated into lipoprotein molecules before passing through the capillary membrane, the rate of transfer across the membrane will depend on the amount of protein leakage. From our present knowledge of the chemical composition *of the alpha and beta lipoproteins* and of the albumin-fatty acid complexes, and of the rates at which fat and protein leave the circulation, it is very improbable that the fat entering the blood stream during digestion is transported across the capillary membrane as lipoproteins

If the fat passes through the pores, as do the proteins, it would seem that it must do so as aggregates smaller than protein molecules; that is the fat cannot be stabilized by protein. Maybe the chylomicrons are broken down to fatty acids which leave the circulation stabilized in some other way, and the albumin-fatty acid complexes are merely incidental

The presence of chylomicrons in the lymph and their probable relation to the plasma chylomicrons must also be considered. A chylomicron of 0 5 μ in diameter is nearly 200 times the size of a pore in the capillary membrane. It is inconceivable that such large amounts of fat in chylomicron form should escape through these pores. It is probable that some such particles may pass through the intercellular cement just as red cells and other large particles do, but not to any appreciable extent Yet this finding may mean that the chylomicrons entering the blood stream during digestion do leave it also in this form If they do, then we must postulate a transfer of fat other than that of lipid insoluble substances through the pores.

Recent evidence suggests that the chylomicrons leave the circulation to a large extent in the liver and spleen and to a much less degree in the other tissues of the body Waddell *et al* (1953, 1954) have shown this in rats after the intravenous injection of artificial fat emulsions. They have also demonstrated that the reticuloendothelial cells play no significant part in the transfer of the fat particles from the blood stream to the liver cells and they conclude that the fat passes freely from the hepatic sinuses into the cells of the liver parenchyma. Our present concept of the liver sinusoids is that they are lined by a continuous endothelium (see Chapter 3), so that the manner in which the chylomicrons escape is still not clear It is possible, however, that they may pass through this endothelium, which we believe is very permeable to the proteins, in the same way as they pass through the lymphatic endothelium which is also very permeable to proteins. As we shall see later in this chapter, chylomicrons very readily enter lymphatic capillaries not only of the small intestine but in all regions of the body, unfortunately, however, we have no knowledge of the precise mechanism of transfer across this endothelial barrier In the normal animal, as we have seen, neutral fat leaving the circulation is nearly all deposited, and only a very small fraction returns to the blood stream in the lymph When the lymph flow from the liver is greatly increased by partially constricting the vena cava just below the diaphragm, however, a considerable amount of fat in chylomicron form enters the hepatic lymph. Fig 41 represents such an experiment in a cat Fatty chyle collected freshly from another cat was injected intravenously and at the same time the inferior vena cava just above the liver was partially obstructed. The rise in pressure in the portal circulation greatly increased the hepatic lymph flow and

the lymph rapidly became milky. The changes in optical density and in the total fatty acid content of the lymph show the enormous increase in chylomicrons in the hepatic lymph which was confirmed by dark-ground microscopy. Obviously under these conditions with a raised

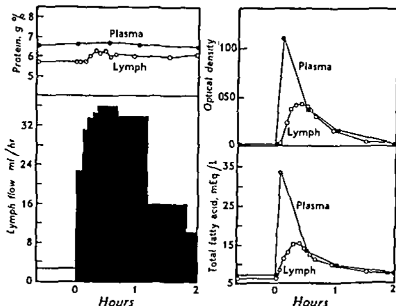


FIG 41 —The composition and flow of lymph from the hepatic lymph duct in a cat under nembutal anaesthesia

The inferior vena cava was partially constricted just above the liver and fatty chyle from another cat injected intravenously

(By courtesy of Mr Bede Morris)

filtration rate, chylomicrons readily pass through the walls of the sinusoids as such, and those that are not taken up by the liver cells pass into the lymph In this experiment 16 per cent of the injected fat was recovered in the lymph in two hours, and similar amounts were recovered in other experiments.

This evidence suggests that fat may leave the circulation in the liver in considerable amounts as chylomicrons without first being broken down to smaller aggregates.

PASSAGE OF LARGE PARTICLES THROUGH THE CAPILLARY WALL

The normal capillary wall may also permit the passage of blood cells and of fine inanimate particles injected into the blood stream. Lymph collected from various lymphatic trunks of the dog always contains some red blood cells and at times considerable numbers. These cells must

have escaped from the blood capillaries in the tissue concerned Clark and Clark (1937) using the rabbit ear chamber technique have actually observed red cells passing through the blood capillary membrane and then through the lymphatic membrane into the lymph stream.

Living particles other than red cells pass through capillary walls without doing any damage. Microfilariae, 40 $\mu \times 5$ μ, readily travel from capillary blood to lymph (Augustine and Drinker, 1935–1936) Field *et al.* (1937) found that rabbits injected intravenously with virulent type III pneumococci show these organisms promptly in leg, neck and thoracic duct lymph In contrast to the very motile microfilariae, pneumococci are not motile at all.

Inanimate particles have also been observed to pass through the capillary endothelium Landis (1934) introduced India ink under a pressure of 50 to 60 mm Hg into mesenteric capillaries in the frog, and noted that "some of the pigment escapes from weak spots in the capillary wall, collecting outside the vessel not uniformly, but in discrete collections Yet afterward blood flows through such a vessel quite normally without undue loss of fluid, in spite of the fact that previously the wall had permitted the passage of India ink, to which even the damaged capillary wall is impermeable." Field and Drinker (1936) injected a graphite suspension into the anterior abdominal vein of frogs The suspension had an average particle size of 1 μ. In less than two hours, free particles of graphite were observed outside capillaries in both the tongue and the web. This extravascular material is not moved through the capillary wall by phagocytes, and it undoubtedly passes through a normal capillary membrane. Similar observations were made with calcite particles, which are easily followed with the dark field microscope. As the calcite moved in the blood stream, a particle could be observed now and again adhering to the capillary wall. If not dislodged, this particle would after a time pass through the endothelium and drift slowly away from the vessel Five to fifteen minutes might elapse from the moment of adhesion until the particle was extravascular and free

All these particles are very much larger than the protein molecules and also much larger than the pores which according to Pappenheimer probably have a radius of 30–45 Å or a diameter of 60–90 Å. A red cell has a diameter of about 80,000 Å while the graphite suspension used by Field and Drinker averaged 1 μ or 10,000 Å in diameter. These particles are therefore 100 to 1,000 times the size of the pores A red cell is elastic and its diameter can be greatly reduced as it squeezes through the capillary membrane. It would seem that a red cell becomes attached to a part of the capillary wall, probably intercellular, and the intracapillary pressure forces the red cell through after considerable distortion of the cell and stretching of the capillary membrane. It is inconceivable that

any individual pore could be so stretched by these particles. The evidence suggests, however, that they pass through the membrane in the intercellular region, Fig. 42 (Chambers and Zweifach, 1940)

Although these large particles will pass through the capillary after first sticking to the wall for some time, the percentage of escaping particles is of course very small compared with the escape of the proteins. The



FIG 42 —Three photographs showing carbon deposited along the endothelial lines of living capillaries in the mesentery of the frog after intravenous injection

(a) An a-v capillary with rapid circulation which accentuated the deposition of carbon in longitudinal striae

(b) and (c) True capillaries with carbon tending to outline the endothelial cells

(From Chambers, R and Zweifach, B W, 1940)

red cell count of lymph varies considerably, but if we assume that thoracic duct lymph contains on an average about 500 red cells/c.mm (see also Chapter 6) then in an adult man in whom the lymph flow per day is about 2 litres, there would be a leakage of 0·2 c.c. blood or about 0 004 per cent of the circulating red cells per day In patients with leukaemia or malignant disease, Bierman *et al.* (1953) have observed larger numbers of red cells than this in the thoracic duct lymph.

PERMEABILITY OF LYMPHATIC CAPILLARIES

We have discussed in some detail the passage of substances and of particles through the blood capillary membrane. The small ions and molecules can return to the blood stream from the tissue fluid as readily as they escaped, but the extravascular protein molecules and particles such as graphite, chylomicrons and red cells may re-enter the blood stream only by way of the lymphatics Although it is generally agreed that the lymphatic capillaries everywhere form a closed space, quite large particles may readily enter these vessels There is no evidence to suggest the site of entrance, but on the analogy of the blood capillaries we should expect absorption of materials to occur through the intercellular region.

Proteins

The fact that extravascular plasma protein enters the lymphatic capillaries and not the blood capillaries has already been discussed. There is a gradient of tension from the tissue space to the lumen of the lymphatic capillary, which explains the mode of entry of fluid into the lymphatics. Two questions concerning the permeability of lymphatic capillaries arise—" Are these vessels of the same permeability throughout the body ? " and " Does the permeability of lymphatic capillaries alter with, say, injury, as does the permeability of the blood capillaries ? "

Regarding the first question it will be seen in Chapter 3 that the lymphatic vessels in different regions of the body are capable of removing a protein-rich fluid such as plasma at very different rates. For example, the lymphatics of the diaphragm will remove free fluid in the peritoneal cavity at a very much greater rate than the pleural lymphatics remove fluid from the pleural cavity or the lung lymphatics from the alveoli or the skin lymphatics from the skin. These observations, however, do not suggest that the permeability of the lymphatic endothelium varies in different regions, but merely that the forces, which are responsible for the entrance of tissue fluid into lymphatic vessels and for the propulsion of the lymph along the collecting channels, vary in these regions. There is no conclusive evidence to indicate that the permeability of the lymphatic endothelium to proteins varies in different tissues

Likewise there is no direct evidence to suggest that in such circumstances as injury proteins will enter lymphatic vessels in any one region more readily than normal. McMaster and Hudack (1932) and Hudack and McMaster (1933), however, observed that various dyes injected into the lymphatic vessels of the mouse's ear or of human skin escape from these vessels into the surrounding tissue fluid more readily after injury caused by mechanical stroking, heat, sunlight or xylol Although these injuries affect the lymphatic capillary wall in such a way as to allow the more ready escape of certain colloidal dyes, the barrier is not so completely broken down as to permit the escape of particulate matter Pullinger and Florey (1935) have also shown that graphite particles once in the lymphatics do not escape as do the much smaller dye molecules These experiments may mean that in injury the extravasated proteins enter the lymphatic capillaries more readily than they would normal vessels

Particles

The ease with which large particles, many times the size of the largest protein molecule, enter the lymphatic vessels makes it difficult to understand how the lymphatic endothelium can become more permeable to

proteins than it normally is. In the skin, where the lymph flow is sluggish, extravasated red blood cells are but slowly absorbed into the lymphatics. In other situations such as the peritoneal and pleural cavities and in the cerebrospinal fluid (see Chapter 3) where the forces bringing the materials into contact with the lymphatic wall and propelling lymph along are much greater, the red cells are very rapidly absorbed. Likewise the smaller chylomicrons readily gain access to the lymphatic vessels when they are present in the extravascular fluid of the intestinal villi as after a fat meal, of the nose as after injection of chyle into the subarachnoid space (Courtice and Morris, 1955), of the diaphragmatic peritoneum as after injection of chyle into the peritoneal cavity (Morris, 1956*b*) It seems, therefore, that if a particle as large as a red cell comes into contact with the lymphatic capillary wall, and provided there is a gradient of tension, the endothelium offers little resistance.

How a particle of this size gets through the membrane is not known. In the tail of the amphibian larva Clark (1909) and Clark and Clark (1926–1927) have shown that when red cells become extravasated, nearby *lymphatic capillaries send out endothelial sprouts which engulf the cells*, Fig. 43. Once inside the vessel, the red cells pass along with the lymph stream This mode of entry of red cells into lymphatics does not apply in the adult organism Clark and Clark (1937) have never observed this sprouting and engulfing of red cells by the lymphatic vessels in mammalian tissues as seen in the rabbit ear-chamber. When lymphatic capillaries lie adjacent to blood capillaries and venules, these authors have observed red cells passing through the wall of both vessels, so transferring from the blood to the lymphatic system There is thus no difficulty for the red cell to enter the lymphatic provided it is brought in contact with the wall.

In the removal of colloids and of particles from the extravascular space by the lymphatics, it is essential that these materials reach the lymphatics and that a gradient of pressure is set up to force them into and along the lymphatic vessels. Motion and massage, as we shall see in Chapter 4, are potent influences upon the entrance into lymphatics of colloidal solutions and of visible particles. Clark (1936) observed that extravasated blood and other detritus were removed from one of his rabbit ear-chambers regardless of the presence or absence of new lymphatics and believed that this observation threw doubt on the theory that lymphatics are essential for the removal of protein from the subcutaneous tissue. But granular material, in such a position as that described by Clark, is not subjected to any influence capable of making it enter a lymphatic. There is no increase in tissue pressure, no motion, nothing which can make the particles become intravascular. Phagocytes may, of course, pick up cells, bacteria, etc., and migrate into blood capil-

laries, though even phagocytes apparently prefer to move back to the blood via the lymphatics. Where particles are not phagocytosed or dissolved, the lymphatic route seems to be the principal one for their removal.

If colloids and particles so readily enter the lymphatic capillaries, why is it that they do not as readily escape? It would seem that in

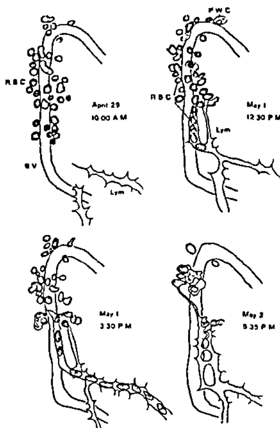


FIG 43.—Successive stages in the lymphatic absorption of extravasated erythrocytes in the frog larva
(From Clark and Clark, 1927)

any one area, if a protein molecule enters a lymphatic capillary, it may a little farther on escape if the gradient of tension here is in the opposite direction. Once the lymph reaches the larger collecting ducts, however, it will, because of valves, be squeezed centrally and will therefore have little chance of again escaping into the tissue fluid In certain instances, however, it is thought that the lymph in its passage along the lymph channels to the blood stream leaks out into the surrounding tissues,

proteins than it normally is. In the skin, where the lymph flow is sluggish, extravasated red blood cells are but slowly absorbed into the lymphatics In other situations such as the peritoneal and pleural cavities and in the cerebrospinal fluid (see Chapter 3) where the forces bringing the materials into contact with the lymphatic wall and propelling lymph along are much greater, the red cells are very rapidly absorbed. Likewise the smaller chylomicrons readily gain access to the lymphatic vessels when they are present in the extravascular fluid of the intestinal villi as after a fat meal, of the nose as after injection of chyle into the subarachnoid space (Courtice and Morris, 1955), of the diaphragmatic peritoneum as after injection of chyle into the peritoneal cavity (Morris, 1956*b*). It seems, therefore, that if a particle as large as a red cell comes into contact with the lymphatic capillary wall, and provided there is a gradient of tension, the endothelium offers little resistance.

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This may happen in the liver lymphatics in causing ascites (p. 188), in the pleural lymphatics during the passage of ascitic fluid along the intra-thoracic lymph channels (p. 188) and in the lung lymphatics in causing pleural effusion (p 193) In all these cases the fluid having entered the lymphatics, where the gradient of pressure was from the tissue spaces to the lumen, now leaves the vessels in regions where the lymph pressure becomes greater than the surrounding pressure.

It would seem, therefore, that as with the blood capillaries the movement of fluid across the membrane is determined by the balance of pressures, so with the lymphatic capillaries the gradient of pressure plays a dominant role. The difference between the two membranes is that while one in offering resistance to the large protein molecules is affected by their osmotic force, the other in readily allowing the passage of such molecules is only affected by the gradient of hydrostatic pressure. What structural difference in terms of " pore " size there is between these two membranes is not known.

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CHAPTER 3

PHYSIOLOGICAL SIGNIFICANCE OF REGIONAL LYMPHATICS

We have seen that whenever capillary filtration is increased in any region, the tissue tension rises; more interstitial fluid enters the lymphatic capillaries and the lymph flow increases. More than this, it is now well established that once particles or large colloidal molecules become extravascular, they can be returned to the blood stream only by the lymphatic route. Although these basic principles apply to the body in general, some tissues, such as the central nervous system, have no lymphatic vessels; the extravascular fluid and protein are here returned to the blood stream in another way. In other tissues the richness of the lymphatic network and the forces propelling the lymph along vary considerably. We also find that in the alimentary tract the extravascular fluid may be greatly modified in amount and composition by absorption of materials through the mucosa from the intestinal lumen The lymph flow will in this case depend not only on the filtration of fluid from the blood capillaries, but also on the rate of intestinal absorption, especially of fluid and of fat

The normal lymph flow as well as the capacity of the lymphatics to remove an excess of extravascular fluid or protein is, therefore, very variable in different regions of the body In this chapter the functional significance of the lymphatic vessels in the individual tissues will be considered in more detail.

Thoracic Duct Lymph

Lymph from all regions of the body finally enters the blood stream at the root of the neck, as described in Chapter 1. Of these final lymphatic channels the thoracic duct is the most striking The flow from this vessel has been studied more than that from any other lymph duct in the body, partly because of its size and of the high rate of flow at rest, which make it relatively easy to cannulate, and partly because of its importance in the absorption of fat from the alimentary tract. We have already shown how in its formation the thoracic duct comes to drain mainly the abdominal viscera, the lower part of the trunk and the lower extremities, vessels from these regions enter the cisterna chyli in the fully developed organism.

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nor from the ducts draining the limbs, while the flow from the right lymph duct in those animals in which it has been measured is small—0·1 ml./kg./hr. in a series of 21 dogs of average weight 21 kg (Courtice, 1951) and 0·15 ml. kg. hr in a series of 10 cats of average weight 3·4 kg (Courtice, unpublished data)

In man lymph has been collected from patients with thoracic duct fistulae. The rates of flow in the two cases quoted in Table 15 were found to be of the same order of magnitude as observed in experimental animals. Crandall, Barker and Graham found that in their patient, a woman of 40 kg with a traumatic thoracic duct fistula in the neck, the basal rates of flow on three experimental days were 1·39, 1·90 and 1·03 ml. kg./hr. with an average flow for all control periods of 1·38 ml./kg./hr. This increased somewhat on drinking water and after a meal, the greatest flow obtained being 5·8 ml./kg./hr observed for a short time soon after a mixed meal, while the lowest flow was 0·6 ml./kg./hr during sleep. Courtice, Simmonds and Steinbeck found in their case, a woman of 50 kg with a postoperative thoracic duct fistula in the neck, a lymph flow of 1·1 ml./kg. hr. in the afternoon, 2½ hours after lunch, 1·6 ml./kg./hr. 2 hours after a heavier meal in the evening and 1·0 ml./kg./hr. in the morning, postabsorptive and under pentothal anaesthesia. Lymph has also been collected in patients after the insertion of a polyethylene cannula into the thoracic duct (Bierman *et al.*, 1953). All these patients were far advanced in the course of malignant disease. In 10 patients lymph was collected for 2 to 13 days continuously. Free flow of lymph was obtained and measured accurately in six of these patients, the volume of lymph collected amounting to 475 to 2,000 ml per 24 hours or 0·4 to 1·2 ml./kg./hr.

This relatively large flow of lymph from the liver and alimentary tract in all animals, whether they live on a high or a low fat diet, and whether they are postabsorptive or not, is one of the most striking features of the lymphatic system. The possible significance of the large turnover of extravascular protein in these regions has already been considered in Chapter 2.

In investigations of the part played by lymphatics in absorption from the alimentary tract, thoracic duct lymph has usually been studied, because this can be collected more easily and with less disturbance to the abdominal viscera than can intestinal lymph. Recently intestinal and liver lymphatics have been separately cannulated to ascertain their relative contributions to thoracic duct flow. Intestinal lymph is usually opalescent or milky, but liver lymph is clear. In dogs, Cain *et al.* (1947) cannulated both the thoracic duct and the main lymphatic duct draining the liver. They collected lymph from these vessels during anaesthesia, and again for varying periods in the unanaesthetized animal after recovery from

The general order of magnitude of lymph flow from the thoracic duct in experimental animals and in man is given in Table 15. During the

TABLE 15

Lymph flow from the thoracic duct in different animals.

Animal		*Lymph flow ml /kg /hr*	*Remarks*	*Author*
Dog	(5)	3·3		Colin (1873)
"	(9)	2·0	Curare	Lesser (1871)
"	(7)	2·5		Zawilski (1876)
"	(15)	1·9	Morphia + ACE	Heidenhain (1891)
"	(20)	1·0	Morphia Fasting	Vinci (1909)
"	(17)	2·7	Morphine + ether	Meyer-Bisch and Gunther (1925*a*, *b* and *c*)
"	(12)	2·4	Chloralose	Yoffey (1932–1933)
"	(16)	1·7	Sodium pentobarbital	Watkins and Fulton (1938)
"		2·2	Local anaesthesia	Petersen and Hughes (1925)
"	(6)	2·2	Nembutal	Courtice (1943)
"	(6)	2·0	Ether	Nix, Mann, Bollman, Grindlay and Flock (1951)
Cat		2·6	Dial and urethane	Adams, Saunders and Lawrence (1945)
"	(4)	0·4	Decerebrate Fasting	Sanders, Florey and Barnes (1940)
"	(6)	1·5	" Fed	Sanders, Florey and Barnes (1940)
"	(7)	2·1	Nembutal	Simmonds (unpublished data)
"	(23)	1·9	Nembutal Fasting	Morris (unpublished data)
"	(30)	2·9	" Fed	Morris (unpublished data)
Rabbit	(14)	2·2	Nembutal + ether	Sanders, Florey and Barnes (1940)
"	(12)	2·3	Nembutal	Morris (unpublished data)
Rat		2·0	Unanaesthetized	Reinhardt (1945)
"	(10)	2·1	Unanaesthetized	Nix, Flock and Bollman (1951)
Horse	(6)	1·9		Colin (1873)
Sheep	(4)	3·0		" "
Bull	(11)	4·0		" "
Cow	(9)	4·9		" "
Goat	(4)	3·8	Nembutal	Courtice (1943)
Man	(6)	0·4–1·2	Unanaesthetized	Bierman, Byron *et al* (1953)
"	(1)	1·4	Unanaesthetized and before meal	Crandall, Barker and Graham (1943)
"	(1)	1·0–1·6	Unanaesthetized and anaesthetized with pentothal	Courtice, Simmonds and Steinbeck (1951)

collection of lymph these animals have all been at rest or under an anaesthetic, in which circumstances there is no appreciable lymph flow from the limbs or from any structure other than the liver and gastro-intestinal tract Although in these experiments there have been many variables such as the type of anaesthetic, the state of the animal's digestion and the duration of lymph collection, the average figures for groups of animals are of the same order, about 2 ml /kg /hr. in non-ruminants and somewhat more in ruminants

This rate of flow is much greater than from all the other collecting ducts put together, since under resting conditions there is usually no spontaneous flow from the cervical ducts draining the head and neck,

nor from the ducts draining the limbs, while the flow from the right lymph duct in those animals in which it has been measured is small—0·1 ml./kg./hr. in a series of 21 dogs of average weight 21 kg. (Courtice, 1951) and 0·15 ml./kg./hr in a series of 10 cats of average weight 3·4 kg (Courtice, unpublished data).

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the operation. Their results are summarized in Table 16. They concluded from these experiments that although there is great variation in

TABLE 16

Mean lymph flows from the hepatic and thoracic ducts in dogs, expressed as ml./hr The figures in brackets give the range.

	Determined alternately		*Determined simultaneously*	
	Liver	*Thoracic duct*	*Liver*	*Thoracic duct*
Anaesthetized	17·8	42 6	17 3	48 2
	(3 0–34 8)	(22 2–101·4)	(9 6–34·8)	(25·8–91·2)
Unanaesthetized	11 0	22 6	4 3	19 8
	(1 8–31 2)	(4 8–84·0)	(3 6–5 4)	(16 8–27 0)
All dogs	13 6	27·6	14·1	42 3

From Cain *et al.* (1947)

the range of normal rates of lymph flow, probably hepatic lymph contributed one fourth to one half of the total volume of lymph in the thoracic duct In this series of animals ether anaesthesia increased the lymph flow.

In another series of experiments with dogs of average weight 15 kg., Nix *et al* (1951) found that under ether anaesthesia the volume of lymph collected from the liver was 14·8, intestine 12·2 and thoracic duct 29 7 ml /hr The liver in these animals, therefore, contributed about half the flow of lymph to the thoracic duct. Unlike the previous experiments, larger volumes of lymph were obtained from dogs recently recovered from anaesthesia.

In all these experiments, the animals whether anaesthetized or not were at rest, so that the lymph flow from the hind limbs would be negligible. When the animal was allowed to walk at 40 yards/minute for a brief time the liver lymph increased by an average of 83 per cent while the thoracic duct lymph increased by 270 per cent. These increases were probably due in part to lymph from the limbs and in part to the increased pressure and massaging effect applied to the abdominal lymphatics by the muscular movement.

In a series of cats under nembutal anaesthesia, Morris (unpublished data) cannulated the hepatic and thoracic ducts, and collected liver and intestinal lymph simultaneously. His results, Table 17, show that under these circumstances the liver on the average contributes about one-third and the alimentary tract two-thirds of the flow in the thoracic duct. These results suggest that the partition of thoracic duct lymph between liver and alimentary tract is much the same in the cat as in the dog. In rats, on the other hand, with fistulae of the intestinal or hepatic duct, Mann and Higgins (1950) found that the hepatic lymph flow was on an average 2·0 ml per day, whereas the intestinal flow was 25·1 ml. per day.

TABLE 17

Lymph flow from the liver and alimentary tract determined simultaneously in the cat.

	Weight Kg.	Lymph Flow ml /hr. Hepatic duct	Thoracic duct
1	3·0	1·1	3·4
2	3·8	0·9	2·0
3	3·7	1·4	2·8
4	2·6	1·2	2·5
5	3·4	1·8	3·2
6	5·1	3·7	4·6
7	3·2	1·7	5·0
8	4·0	1·4	5·6
9	2·6	1·3	2·2
10	3·6	2·7	5·8
Mean	3·5	1·7	3·7

By courtesy of Mr Bede Morris

In these experiments, therefore, in which the animals had a standard mixed diet and 0·2 per cent NaCl to drink *ad lib*, the hepatic lymph contributed less than 10 per cent of the thoracic duct flow It is probable, therefore, that the relative volumes of lymph contributed by the liver and by the intestines to the thoracic duct vary in different animals.

THE ALIMENTARY TRACT

Lymph has not been collected separately from the stomach, small intestine and colon, so we do not really know the relative volumes which these different organs contribute to the cisterna chyli and thoracic duct. It seems likely, however, from the experiments just described, in which the intestinal vessel cannulated drained mainly the small bowel, that the profuse lymph flow from the alimentary tract comes largely from this region Here, during digestion, considerable volumes of fluid and materials are absorbed through the mucosa and come into contact with the lymphatic and blood capillaries in the villi The lymph in the intestinal lymphatics is, therefore, not merely concerned with the removal of extravascular protein which has escaped from the blood capillaries, but is modified by the state of digestion at the time The lymphatic capillaries here have a special function, the absorption of fat, but even in herbivora and especially in ruminants, whose diet is consistently very low in fat, the intestinal lymph flow is high. In contrast to the lymphatic vessels of the small intestine, those of the oesophagus, stomach and large intestine are concerned mainly with the removal of extravascular protein as in any other tissue; for, through the mucosa of these organs little absorption takes place.

General arrangement of lymphatics in the alimentary tract

Oesophagus. Lymphatics are found in the mucous membrane and in the submucosa of the oesophagus. They pass to nodes scattered in the deep tissue of the neck and to nodes in the posterior mediastinum. Absorption from the surface of the oesophagus is probably extremely slight (Webb, Mullenix and Dragstedt, 1932), and the lymphatics are concerned solely with material escaping from the blood capillaries. Since the submucous lymphatics extend into the muscular coat, oesophageal contractions must have a vigorous effect in forcing contained fluid towards the lymph nodes

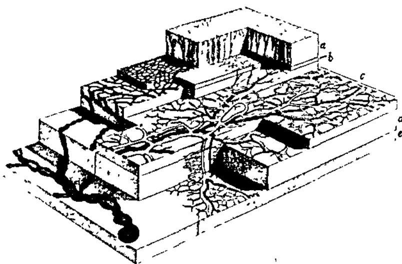


FIG 44 —Diagrammatic reconstruction of the blood vessels and lymphatics in the wall of the stomach
a, mucous coat, *b*, muscularis mucosae, *c*, submucous coat, *d*, circular muscle, *e*, longitudinal muscle
(From Mall, 1896)

Stomach The stomach is richly supplied with lymphatics. They originate as many blind projections among the tubular glands, and do not extend so near the surface as the network of blood capillaries. Fig. 44 is a diagrammatic reconstruction of the blood vessels and lymphatics in the dog's stomach (Mall, 1896) The lacunar projections of the lymphatic capillaries join a net on the muscularis mucosae. From this plexus, many branches pass at right angles through the muscularis mucosae to form a second plexus lying in the submucosa. The vessels of this submucosal plexus are large and irregular and have many valves. The valves prevent the lymph from going back into the mucosal vessels, so that during gastric contraction, the lymph can only be pushed onwards

to the cisterna chyli. From the submucous collecting branches large vessels arise and perforate the circular muscle coat to communicate with the intermuscular plexus which ultimately drains to lymph nodes on both curvatures of the stomach. Absorption from the mucosa is slight. Alcohol, traces of sugar and possibly a little water are taken up, but the lymphatics are almost entirely concerned with the extra-vascular circulation of protein.

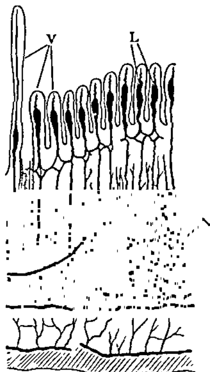


FIG 45—Arrangement of lymph vessels in the wall of the small intestine of the dog

L Lacteals
N Lymphoid nodules The nodule on the left is shown in section while that on the right is viewed from the surface, with lymph vessels coursing over it
V Villi Unless the villus is put on the stretch and elongated, as on the left, its lacteal usually shows a characteristic spiral twist near the apex For the most part, the columnar epithelium has been omitted

(Redrawn after Mall, 1888)

Small intestine The mucosal surface of the small intestine is greatly increased by the formation of circular folds and by the villi This arrangement is especially suited for the absorption of materials from the lumen The villi are outgrowths of the mucous membrane and in man are finger-like processes having a length of 0 5 to 1 5 mm They cover the entire surface of the mucosa of the small intestine, giving it a velvety appearance. They are most numerous in the duodenum and jejunum where there are 20 to 40 per sq mm. while in the ileum there are 15 to 30 per sq mm These long finger-like villi are also present in the dog, cat, goat, fox, eagle, pigeon and hen In the rat, rabbit, mouse, guinea-pig and squirrel, however, the villi are flat and leaf-shaped (cf Verzar and McDougall, 1936) In the centre of each finger-form villus is a lymph capillary—the lacteal—which begins blindly under the epithelium covering the tip of the villus The lumen of these lacteals, when distended, is considerably larger than that of the blood capillaries In the rat, on the other hand, with flat, leaf-like villi, Ranvier (1896) describes three, four, five or more lymphatic vessels each ending in a cul-de-sac and often anastomosing by transverse or oblique branches

The central lacteals at the base of the villi anastomose with the lymphatic capillaries between the glands which form a plexus on the inner surface of the muscularis mucosae, Figs 45 and 46. Branches of this plexus, provided with valves, penetrate the muscularis mucosae and form in the submucosa a plexus of larger vessels. This plexus also receives tributaries from the dense network of large, thin-walled lymphatic capillaries surrounding the solitary and aggregated follicles. Vessels from the submucosal plexus communicate with the intermuscular plexus which drains through mesenteric channels into the mesenteric lymph nodes from which the lymph enters the cisterna chyli.

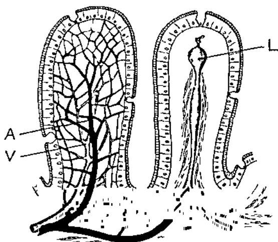


FIG 46—Schematic diagram to indicate the arrangement of blood and lymph vessels in an intestinal villus

To avoid confusion, the villus on the left is shown only with its blood vessels, while on the right only the lacteal has been included The central artery (*A*) passes towards the apex of the villus, and breaks up into a spray of capillaries which lie immediately under the mucous membrane before they turn in towards the central vein (*V*) The lacteal (*L*) is further away from the intestinal epithelium than the blood capillaries

(Redrawn and modified from Maximow and Bloom, after Mall)

It is now thought that the absorbing surface is increased not only by the folds and the villi, but also by the conformation of the free striated border of the epithelial cells of the small intestine. Although various conceptions of the nature of this free border have been held (cf Baker, 1942, 1951), electron micrographs show that it is composed of minute processes projecting vertically into the lumen (Granger and Baker, 1950). These processes, on an average 0·62 μ in length and 0·08 μ in diameter, are packed closely together and it has been estimated that they increase the absorptive surface by about 30 times.

Large intestine. The lymphatics of the large intestine and rectum are extremely numerous and form plexuses in the mucosa, passing eventually through the muscle layer. Although water is absorbed freely in the

large intestine, the function of the lymphatics would seem to be mainly concerned with the return of the extravascular protein to the blood stream.

Role of lymphatics of small intestine in absorption

An account of the mechanisms involved in the absorption of materials through the intestinal mucosa is beyond the scope of this monograph. Reference should be made to such works as Verzar and McDougall (1936), Bloor (1943) and Höber (1945). We are here interested mainly in the relative roles of the lymphatic and of the blood capillaries in taking up substances once they have passed through the epithelium.

Effect of villus movement on lymphatic absorption. The observations that the villi are quiet in the fasting animal, but move rhythmically during the digestion of foodstuffs suggest that villus movement is of biological significance in absorption. In the subepithelial framework of the villus are strands of smooth muscle which arise from the muscularis mucosae and are arranged parallel to the axis of the villus around the central lacteal. On contraction of these muscle fibres the villi are shortened, and on observation the rhythmical movement up and down suggests a pumping mechanism. The normal rate of this villous retraction during activity varies under different circumstances. In those animals that do not have long, finger-like villi, there is no actual contraction of the villi, but circular contractions of the underlying muscularis mucosae press the villi together and so possibly produce the same result (cf. Verzar and McDougall, 1936) Many think that the rhythmical contractions of the villi increase absorption, for in general the greater the movement of the villi, the greater the rate of absorption (cf. King and Arnold, 1922; King, Arnold and Church, 1922; Kokas and Ludány, 1930, 1933, 1937, 1938; Magee and Reid, 1931). This view of the function of the villi is opposed by Wells and Johnson (1934) who conclude that the transfer of fluid through the gut wall, both absorption and secretion, is by physical forces and is regulated primarily by vascular reactions, local and general. They found no correlation between the motor activity of the villi and the rate of passage of fluid through the gut wall.

The above experiments have been concerned mainly with the absorption of water and readily diffusible water-soluble molecules. In such cases it is quite possible that other factors affecting blood flow and the balance of pressures within the villus are more important than the phasic pressure changes which accompany villus movement. However, the absorption of those substances which are removed only by the lymphatics must depend upon the efficient emptying of the lacteals, and the rhythmic retraction of the villi would seem to compress the lacteals and force the lymph on into the collecting channels Kunigés and Otto (1936) by

direct measurement found that the pressure in the lacteals was always less than that in the arterioles and capillaries; on an average 37·3 mm. Hg in arterioles, 31·5 mm. Hg in the capillaries and 24·5 mm. Hg in the lacteals. When the villus contracts, Wells and Johnson observed that the blood flow often immediately ceases, suggesting that the tissue pressure is raised above that of the arterioles. This rise of tissue pressure would empty the lacteals, the lymph passing on into more distant collecting vessels.

Histological examination of the gut wall during fat absorption reveals that the central lacteals are empty in the contracted villi and full in others (cf. Verzar and McDougall, 1936) This indicates that in the case of fat, which, as we shall see, is probably almost entirely absorbed by the lymphatics, the contracting villi have a pumping action; the fat enters the lacteals during relaxation and is propelled along the lymphatics during contraction.

Lymph flow during absorption of fluids. It is well known that in anaesthetized animals thoracic duct lymph flows more rapidly after a meal Yet, until recently, it has not been possible to study with accuracy the mechanism of this increased flow. Anaesthesia affects digestion, absorption and gut motility, and attempts to collect lymph from conscious animals have been foiled by rapid clotting in the cannula The use of plastic tubing, such as transflex or polythene, in which clotting is delayed, has made it possible to collect thoracic duct lymph in unanaesthetized animals. In particular the technique devised by Bollman, Cain and Grindlay (1948) in the rat has been used extensively in recent years.

Using the unanaesthetized rat with a thoracic duct fistula several workers have investigated the lymph flow after the introduction of various fluids and foodstuffs into the stomach. Simmonds (1954) gave single doses of 5 ml of water, 0 9 per cent sodium chloride, 10 per cent serum albumin in water or 10 per cent glucose by stomach tube, and found that in all cases the lymph flow rapidly increased while the protein concentration in the lymph fell, Fig 47. This suggests that some of the absorbed fluid passed directly into the lacteals. Of the 5 ml of fluid given, about 2 ml or 40 per cent was accounted for by the increase in lymph flow With water and a hypotonic solution such as 10 per cent serum albumin in water, the increase in lymph flow was very rapid and lasted only a short time, about half an hour. On the other hand with isotonic sodium chloride or hypertonic glucose, the rise in lymph flow was slower and more prolonged. The total protein output in the lymph, in mg./hr., did not alter materially in the experiments with water or 10 per cent albumin The absorption of the water was so rapid that little or no extra protein was added from the blood capillaries. With

saline and glucose, however, the absorption of water was slower and a little extra protein did filter or diffuse from the plasma and pass into the lymph; but the extra fluid came mainly from the absorbed water. Borgstrom and Laurell (1953) obtained similar results after the ingestion of 0·9 per cent NaCl and 5·5 per cent glucose.

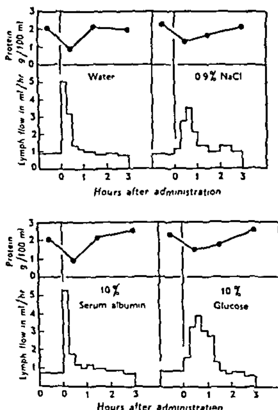


FIG 47—The effects of 5 ml of water, of 0·9 per cent sodium chloride, of 10 per cent serum albumin or of 10 per cent glucose, introduced into the stomach in a single dose, on the flow and protein concentration of lymph collected from a polyethylene cannula in the cisterna chyli of the unanaesthetized rat

(From Simmonds, 1954)

If, instead of a single dose, the fluid is given continuously over a [illegible]

[illegible]

increase in thoracic duct lymph flow with small urinary output, whereas 5 per cent glucose alone produces a small increase in lymph flow and

a larger urinary output (Glenn *et al.*, 1949). Rats with a thoracic duct fistula drink a great deal more if 0·8 or 1 per cent sodium chloride solution is provided than they do with free access to water. The large amount of fluid ingested leaves the body in the thoracic duct lymph, Table 18 (Shrewsbury and Reinhardt, 1952)

TABLE 18

Lymph and urine output in rats with thoracic duct fistulae and with either 1% NaCl or water to drink *ad lib.* 4 days' collection of lymph

	1% NaCl	*Water Intake*
Number of animals	8	6
Initial body weight g	199	235
Final body weight g	145	188
Fluid intake ml	686	108
Lymph output ml	557	59
Urine output ml	69	36
Lymph protein g	3 2	1·9
„ „ g %	0 6	3 4
Plasma protein initial g %	5 42	5 59
„ „ final g %	3 29	5·21

From Shrewsbury and Reinhardt (1952)

When the same amount of saline or of water is given by continuous drip transfusion into the stomach, the lymph output rises continuously with saline, but with water the rise slows and the output then falls as the urinary output increases, Fig 48 In the early stages after cannulation of the thoracic duct in a rat, it is easier to keep the lymph flowing and so prevent clotting by allowing the animal to drink 0·8 or 1 per cent sodium chloride solution. In these cases of continuous prolonged ingestion of fluid, the total protein in the lymph is greatly increased with lymph flow although the concentration falls markedly. Some of the fluid diluting the lymph proteins and increasing the lymph flow must come directly from the lumen of the intestine, but in addition a considerable amount is produced by the increased filtration from the capillaries (Simmonds, 1954; Kim and Bollman, 1954).

Lymph flow during absorption of foods. One of the earliest observations on lymphatics was the milkiness of the lacteals in a dog after a fat meal, made by Asellius in 1622. In more modern times, the lymph has been collected after a fat meal and the absorbed fat examined. It is now generally recognized that a large fat meal causes an increased flow of milky lymph from the intestinal lymphatics. This may be observed, for example, in a rat with a thoracic duct fistula after introducing 0·5 ml. of olive oil into the stomach (Fig 49). The lymph flow increases as a rule two or three times, but in some cases even 5 to 10 times, the resting level and the increased flow is maintained for several

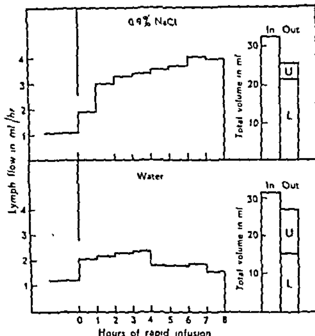


FIG 48—The effect on the hourly production of thoracic duct lymph of 0.9 per cent sodium chloride solution (upper diagram) and water (lower diagram) introduced into the stomach at a uniform rate of 4 ml/hr for 8 hours in the unanaesthetized rat

Each lymph flow diagram is followed by the total fluid exchange in the 8-hour period. U = urine volume in ml. L = lymph in ml.

(From Simmonds, 1954)

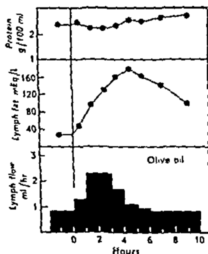


FIG 49—The effect of 0.5 ml olive oil, introduced into the stomach in a single dose, on the flow and composition of lymph from the thoracic duct of the unanaesthetized rat

(From Simmonds, 1954)

hours This, however, is a large dose of fat—much more than is ever taken by man or animals under normal circumstances.

To investigate the effect of digestion and absorption of more physiological diets on lymph flow, Simmonds (1955*b*) fed to rats with thoracic duct fistulae 1 g of dry skim milk powder or of full cream milk powder or of standard rat nuts without giving any fluid. In other experiments he gave 5 ml of 10 per cent (i.e. 0·5 g.) skim milk or full cream milk (Fig. 50). It became evident from these experiments that when dry food was given, the lymph flow was increased only when fat was being absorbed. When water was given, too, the absorption of the fluid tended

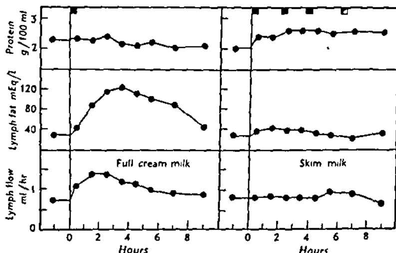


FIG 50 —The effect of 1 g of full cream milk powder and of skim milk powder, fed in the dry state, on the flow and composition of lymph from the thoracic duct of the unanaesthetized rat

(From Simmonds, 1955)

to mask the changes in lymph flow produced by the fat. These experiments would suggest, therefore, that intestinal lymph flow is appreciably increased during digestion only by absorbed water or by absorbed fat, proteins and carbohydrates eliciting little or no increase in lymph flow

Absorbed fluid seems merely to dilute the extravascular fluid in the intestinal mucosa. There is little increase in the capillary filtration of protein. The swollen extravascular space then shrinks to its normal size due partly to an increased flow of lymph of low protein concentration and partly to absorption of protein-free fluid by the blood stream. The latter process restores tissue fluid and lymph protein concentrations to their preabsorptive values. During fat absorption, on the other hand, there is not only an expansion of the extravascular space but also a great increase in the turnover of extravascular protein. The lymph flow

increases considerably but the protein concentration in the lymph does not fall. This must mean that filtration from the blood capillaries is increased and that the extravascular space later shrinks *pari-passu* with the removal in bulk of tissue fluid and protein by the lymphatics (Simmonds, 1955*b*). The mechanism whereby capillary filtration of both fluid and protein are increased during fat absorption but not during protein and carbohydrate absorption is not clear. It may be associated with motility of the gut, or with hyperaemia of the gut. All we know at present is that when it becomes necessary for fat to be absorbed into the lacteals, there is increased formation of extravascular fluid to carry it away in the lymph.

In the human being Crandall, Barker and Graham (1943) studied the effect of 50 ml. of olive oil and of a mixed meal consisting of boiled chicken, cauliflower, ½ glass of buttermilk, white bread and a bowl of vegetable soup The lymph flow increased in both cases as shown in Table 19 As with experimental animals the lymph flow after olive oil is much more prolonged than after a low fat meal consisting of a good deal of fluid; whereas the flow was still much above the resting level 4 hours after drinking the olive oil, it rose sharply and returned to the initial level in about 1 hour after the meal.

TABLE 19

The effect on the thoracic duct lymph flow in man, of the ingestion of 50 ml olive oil and of a mixed meal

50 ml Olive oil Time	Lymph flow ml /min	*Mixed meal* Time	Lymph flow ml /min
9 33	0 75	12 45	0 99
9 48	0 81	1 00	1 47
10 03	0 81	1.15	1 35
10 18	0 73	1.25	1 26
10 33	0 60	Meal	
11 03	0 47	2 05	3 90
50 ml olive oil		2 20	3 55
11 23	0 58	2 35	2 25
11 53	0 64	2 50	1 51
12 23	0 36	3 05	1 25
1 01	1 00		
1.31	1 50		
2 01	1 60		
2 31	1 73		
3 01	1 09		
3 31	1 51		

From Crandall, Barker and Graham (1943)

Transfer of fat in the lymph The mode of transfer of fats through the intestinal mucosa to the underlying tissue spaces has been the subject

of debate for nearly a century and is still controversial. Up to the end of the last century it was thought that fats after emulsification by the bile, passed through the epithelial cell as fine particles and appeared in the lymph as neutral fat. In 1900 Pfluger opposed this theory; he postulated that ingested fat was broken down by lipase to fatty acids and glycerol which entered the epithelial cells as sodium soaps and glycerol, to be resynthesized chiefly to neutral fat (cf. Verzar and McDougall, 1936, Verzar, 1948). In recent years Frazer has put forward an hypothesis which combines these two theories. He suggested that a portion of the fat is hydrolysed, the resulting fatty acid being absorbed mainly into the portal blood, while the remaining portion is absorbed as a fine emulsion of neutral fat and passes into the lymph. This has been called the "partition hypothesis" (cf. Frazer, Schulman and Stewart, 1944; Frazer, 1943*a* and *b*, 1946, 1948, 1952).

It is not proposed to enter into the controversy concerning the mode of passage of the ingested triglycerides through the mucosal cells. Reference should be made to the papers and monographs cited. Once the lipid is through the mucosa, however, its fate becomes closely associated with the lymphatic system. In 1891 Munk and Rosenstein found in a patient suffering from elephantiasis with a chylous fistula in the leg that as much as 60 per cent of ingested fat could be recovered from the fistula. For over half a century it was then assumed that approximately 60 per cent of ingested fat is absorbed into the lacteals while the remaining 40 per cent goes by some other route, presumably the portal blood stream.

Apart from occasional cases of fistula in man, lymph has usually *been collected experimentally from animals under anaesthesia when the* digestion and absorption of fat are probably decreased For example, Eckstein (1925) fed 5 to 100 g. of olive oil to anaesthetized dogs and collected in the lymph during the next 6 to 21 hours only 4 to 21 per cent of the amount of fat administered; Morris (1954) introduced 3 ml./kg. of olive oil into the stomachs of anaesthetized cats and rabbits and found 6 to 21 per cent of this amount in the thoracic duct lymph of the cats and only ½ to 2 per cent in the lymph of the rabbits during the ensuing 8 to 10 hours. By the use of polyethylene catheters in unanaesthetized animals and of radioactive isotopes to label the ingested lipid practically all the ingested lipid can be recovered in the lymph in rats. Bloom, Chaikoff, Reinhardt, Entenman and Dauben, 1950, in one series collected 70 to 92 per cent and in a later series of experiments this group of workers was able to recover in the lymph 91–97 per cent of absorbed C^{14} as lipid C^{14} when palmitic acid labelled with C^{14} was given as triglyceride (Bloom, Chaikoff, Reinhardt and Dauben (1951)). These and other similar experiments suggest that practically all ingested fats, which in normal circumstances are ingested mainly as the triglycerides

of palmitic, stearic and oleic acids, enter the lymphatics of the intestine after absorption through the mucosa.

[illegible]

amounts produced no milkiness of the intestinal lymphatics and no significant change in the chyle. Many direct findings fail to support these observations after feeding fatty acids. That fed fatty acids were absorbed into the lymph as triglycerides was shown long ago by Munk and Rosenstein (1891) and Argyris and Frank (1913) and in more recent years by Freeman and Friedemann (1935), Freeman and Ivy (1935), Bollman *et al.* (1950), Bloom *et al.* (1950), Bergström *et al.* (1950, 1952, 1954), Borgstrom (1951), Reiser and Bryson (1951), and Simmonds (1955*a*) By cannulation of the thoracic duct and collection of the intestinal or thoracic duct lymph overwhelming evidence has been obtained to show that long chain fatty acids, whether fed as the free acid or as the triglyceride, are transported from the intestinal mucosa almost entirely in the lymph and mainly as triglyceride. As we have seen in Chapter 2, lipids in the tissue fluid in the form of chylomicrons readily enter lymphatics in any region of the body. There is evidence, however, that some of the short chain fatty acids do enter the portal blood stream. Bloom, Chaikoff and Reinhardt (1951) and Chaikoff *et al.* (1951) showed that whereas absorbed stearic, palmitic and pentadecanoic acids can be recovered almost quantitatively in the intestinal or thoracic duct lymph, only 60 to 80 per cent of absorbed myristic acid, 15 to 55 per cent of lauric acid and less than 20 per cent of decanoic acid can be recovered in the lymph. Kiyasu, Bloom and Chaikoff (1952) determined the fatty acid C^{14} in the plasma of the portal and inferior vena caval blood 30 to 190 min after giving fat labelled with C^{14}. When palmitic acid was given the ratio portal vein fatty acid C^{14}/I.V C. fatty acid C^{14} was 0 91–1·2 while this ratio varied from 1·3–9·7 after feeding decanoic acid These findings are consistent with the view that short chain fatty acids are transported mainly by the portal pathway and long chain saturated fatty acids via the lymph.

In the postabsorptive animal most of the lipids are carried in the plasma as lipoprotein (Chapter 2). The plasma phospholipid in these circumstances is formed mainly in the liver (Fishler *et al.*, 1943) although other tissues such as heart, skeletal muscle, kidney, small intestine and lung do form phospholipids from administered labelled palmitic acid (Goldman, Chaikoff, Reinhardt, Entenman and Dauben, 1950) It has been recognized for a long time that during fat absorption there is an increase in the phospholipid concentration in the intestinal lymph (cf. Verzar and McDougall, 1936) Bollman *et al.* (1950) found in

unanaesthetized dogs with thoracic duct fistulae that the phospholipid increased as much as threefold with the maximal increases found in neutral fat. As the total fat in the lymph increased, however, the fraction due to phospholipid decreased (Table 20). They also showed that there

TABLE 20

Total lipids and their distribution in thoracic duct lymph of the dog after a fat meal.

Total lipids mg %	*Neutral fat* % *of total*	*Phospholipid* % *of total*	*Cholesterol* % *of total*
437–1000	51·3	30·5	18·1
1001–2000	71·5	19·9	8·1
2001–3000	79·5	14·9	5·7
3001–4000	84·1	12·0	3·7
4001–5000	85·9	11·1	2·8

From Bollman, Flock, Cain and Grindlay (1950)

was no increase in lipid in the hepatic lymph during fat absorption. Bloom *et al.* (1951) fed rats labelled palmitic acid as triglyceride and found of the 91–97 per cent of the absorbed labelled fat recovered in the lymph only 2·3–3·7 per cent of the labelled fatty acids had been incorporated into phospholipid. These experiments and others (cf Bergstrom *et al.*, 1950, 1952; Borgstrom, 1951, 1952*a* and *b*, 1953) therefore suggest that phospholipid is formed in the intestines during fat absorption but that quantitatively it is not an important transport form for absorbed fat.

Morris (1954) determined the interrelationships of the lipid fractions in thoracic duct lymph of the cat, rabbit and rat after the ingestion of triolein During absorption the concentrations of all lipid fractions increased in parallel, Fig. 51, and the results show that the phospholipid was produced in the intestines since at all times the level in the lymph was greater than in the plasma; that relative to the total postabsorptive phospholipid in the plasma, the quantities of phospholipid in the lymph may be considerable, but that relative to the total amount of fat transported in the lymph the phospholipid fraction is small. When the fatty lymph collected during fat absorption is centrifuged at 20,000 r.p.m., the chylomicron layer will float to the top It was found by this procedure that all the increase in the phospholipid was in the chylomicron layer while the lipid concentrations in the clear layer below were the same as in the lymph before fat administration. As we have mentioned in Chapter 2 the electrophoretic patterns on the fluid after the separation of the chylomicron layer show that during fat absorption there is no significant alteration in the lipoproteins.

There is also an increase in the cholesterol output in the thoracic

duct lymph when fat is absorbed (Fröhlicher and Sullmann, 1934; Brockett, Spiers and Himwich, 1934; Freeman and Ivy, 1935; Bollman and Flock, 1951; Morris, 1954) Like phospholipid, this is only a small fraction of the total lymph lipid and it seems likely that this cholesterol is associated with the neutral fat in the structure of the chylomicrons. Most of the absorbed cholesterol in intestinal lymph is in the

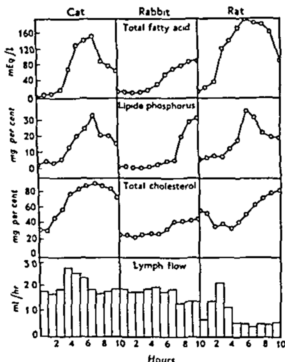


FIG 51 —The flow and lipid composition of thoracic duct lymph after introducing 3 ml /kg olive oil into the stomachs of cats and rabbits, and 1 ml olive oil into rats (From Morris, 1954)

form of esterified cholesterol which is in marked contrast to the small percentage in the intestinal mucosa. This indicates that large amounts of cholesterol are esterified by the intestinal mucosa By feeding labelled cholesterol to unanaesthetized rats with a thoracic duct fistula, Chaikoff *et al* (1952) showed that all absorbed cholesterol enters the intestinal lymphatics, and Siperstein, Chaikoff and Reinhardt (1952) showed that bile was an obligatory requirement for the passage of cholesterol from the intestinal tract to the lymph.

It would seem, therefore, that under normal circumstances ingested fat after passing through the intestinal mucosa enters the lacteals in the

unanaesthetized dogs with thoracic duct fistulae that the phospholipid increased as much as threefold with the maximal increases found in neutral fat. As the total fat in the lymph increased, however, the fraction due to phospholipid decreased (Table 20). They also showed that there

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There is also an increase in the cholesterol output in the thoracic

and probably very permeable membrane. Two types of cells make up this tenuous membrane. The first is an undifferentiated endothelial element, extending along the surface of the liver cells, sometimes closely applied to them, sometimes with a narrow cleft between. The cytoplasm of these cells is often continuous with that of the second type of cell, the conspicuous stellate cells of Kupffer, which—in contrast to the

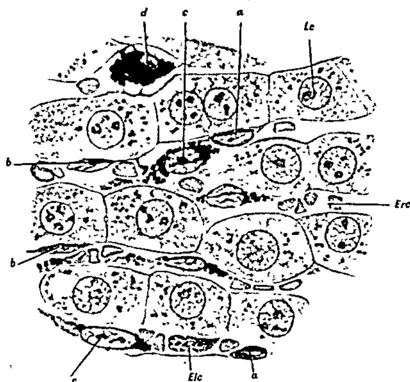


FIG 52—Liver of rabbit, injected intravenously with India ink

Lc, liver cell, *Erc*, erythrocyte in lumen of sinusoid, *Elc*, eosinophil leukocyte, *a*, cells of wall of sinusoid in resting condition, *d*, stellate cell of Kupffer, *b* and *c*, transitions from *a* to *d* Haematoxylin-eosin-azure II stain

(From Maximow and Bloom, *A Textbook of Histology*, W B Saunders Co, Philadelphia, 1952)

simpler endothelial lining cells—are markedly phagocytic and stain with vital dyes. The Kupffer macrophages sometimes possess endothelial processes which seem to extend like lacework across the sinusoids, an arrangement very advantageous for making contact with foreign particulate matter in the blood (see Fig 52).

In the mammalian liver, the relation of blood capillaries to lymph vessels has been worked out by Lee (1923) This author found that

form of chylomicrons which consist mostly of triglycerides but which contain also some cholesterol and phospholipid. The lipid present as lipoproteins in the intestinal lymph comes mainly from the plasma as capillary filtrate; it is small in amount compared with the amount of fat that may be present as chylomicrons and does not alter appreciably during fat absorption

Absorption of protein molecules and of particles. The problem of the direct absorption of unchanged proteins from the bowel has much importance in clinical medicine. It seems likely that any protein which passes unchanged through the intestinal mucosa enters the lymphatics and not the blood capillaries. Alexander, Shirley and Allen (1936) fed dogs egg albumin by stomach tube and found it at once in the thoracic duct lymph but not in the portal blood. They concluded that lymphatic absorption was necessary for systemic dosage with egg white. With regard to proteins of larger molecular size, the feeding of colostrum to the newborn of many species is followed by the appearance of certain of the colostrum proteins in the blood serum. This capacity to absorb large amounts of unchanged protein disappears within 24–48 hours of birth. Comline, Roberts and Titchen (1951) have shown that in newborn calves these proteins, which are associated with the antibodies of the colostrum and have the same electrophoretic mobility as the adult serum γ globulins, are absorbed unchanged into the intestinal lymphatics *and not into the blood stream*

In the case of particles such as bacteria, it is generally thought that injury to the mucous membrane is necessary to permit penetration; but it seems certain that such organisms as the typhoid bacillus must penetrate the normal epithelium and proliferate in the mucosa, particularly in the lymphoid tissues. Once resident in the mucosa, these bacilli—with little doubt—will after a time be found in the lymph, and will very possibly reach the blood largely via the thoracic duct lymph. In recent years it has been thought that the virus of poliomyelitis enters the body through the alimentary tract Although it has not been proved directly, it would seem reasonable to postulate that this virus passes into the lymphatic vessels in which it is conveyed to the blood stream (see also Chapter 7).

Liver

General anatomical arrangement

It is now generally believed that no direct communications exist between the portal blood and the liver cells, but that the cells are separated from the blood in the sinusoids of the lobules by an extremely delicate

towards the porta hepatis where they enter the hilar lymph glands From these lymph glands efferent vessels pass in the lesser omentum to the cisterna chyli This is probably the main flow of lymph from the liver, and in collection of liver lymph, it is one of these vessels that is usually cannulated (cf. Cain *et al*, 1947) All the liver lymph, however, does not pass by this route. Some flows through vessels which traverse the falciform ligament and the diaphragm anteriorly to enter substernal lymph nodes, some passes through vessels accompanying the hepatic veins and enters the lymph nodes lying adjacent to the upper end of the inferior vena cava, while some drains by communicating channels with the vessels of the gall-bladder. The general arrangement of these pathways is shown diagrammatically in Fig 53. It must be remembered, therefore, that by ligating or cannulating the main lymph channels emerging from the porta hepatis not all the liver lymph is prevented from entering the circulation or is collected in the cannula.

Flow and composition of liver lymph

It has long been thought that the liver was responsible for furnishing a large part of the thoracic duct lymph. Although Markowitz and Mann (1931) in experiments in which they collected thoracic duct lymph from dogs, found no diminution in the volume of this lymph after ligation of the periportal lymphatics or after liver removal, experiments described on page 123 suggest that the lymph from the liver contributes a considerable part of the thoracic duct flow in dogs and cats but perhaps less in rats No information is available concerning the amount of lymph that flows into vessels other than those emerging from the liver at the porta hepatis

The permeability of the endothelial lining of the liver sinusoids has generally been regarded as high Starling (1909) measured the protein concentration of liver lymph collected from large vessels in the lesser omentum, and found nearly as much protein as in the circulating blood Field and her associates (1934–1935) collected liver lymph from 4 dogs, the protein concentration averaging 5·32 per cent, while in a series of 10 cats McCarrell, Thayer and Drinker (1941) found that on an average the protein concentration was 5 74 per cent in the plasma and 5·58 per cent in the liver lymph. The flow and composition of lymph collected from the liver and from the intestines in a series of dogs and cats are shown in Table 21 (overleaf). It is evident that in these animals the liver contributes a very high proportion of the extravascular protein in thoracic duct lymph.

In the liver, therefore, there is a low filtering head of pressure, a high lymph flow and the highest protein concentration of lymph from any part of the body. These factors would suggest a high permeability

"lymph vessels formed a rich plexus in Glisson's sheath and liver capsule, extended up to but not within the liver lobule, formed many anastomoses between portal units, and established abundant communications with similar vessels in the walls of hepatic veins". One must consider that fluid and solutes leave the liver sinusoids in the lobule

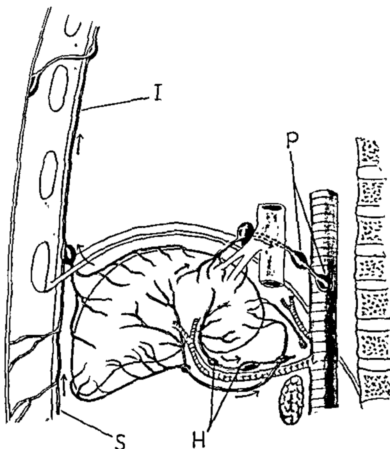


FIG 53 —Schematic diagram of lymphatic drainage of liver The arrows indicate the direction of the lymph flow

I [illegible]
P [illegible]
H [illegible]
S [illegible]
[illegible]

and are, for a time, tissue fluid between the liver cells and the walls of the sinusoids. This fluid in so far as it is removed as lymph, remains extravascular until it reaches lymph capillaries at the periphery of the lobule and until these capillaries, in their turn, eventually deliver it to trunk vessels, such as those in the lesser omentum.

The lymphatic vessels in Glisson's capsule ultimately pass mainly

Watson and Bale, 1951; Miller and Bale, 1954; Miller, Bly and Bale, 1954) It is not certain, however, how these newly-formed proteins enter the blood stream.

It would seem that any protein formed by the liver cells would pass into the space between the cells and the endothelial lining of the sinusoids where it would mix with the extravascular fluid. Then with this fluid and the protein escaping from the sinusoids it would pass to the periphery of the lobules, where it would enter the lymphatic capillaries and be carried away by the liver lymphatics. If newly formed protein could pass from the liver cell through the endothelium of the sinusoids directly into the blood stream, it is hard to understand why lymphatics in the liver are necessary at all. For, if newly-formed plasma protein in the fluid spaces can enter the blood stream directly, there seems no reason why extravascular protein should not return by this route.

Experiments by CoTui, Barcham and Schafiroff (1944) provided evidence to support the view that newly-formed proteins mainly entered the blood by way of the lymph They ligated the thoracic ducts in one group of dogs, bled them and followed the concentration of protein in the blood; they compared the results of those obtained after haemorrhage in dogs with thoracic duct intact. The level of protein in the blood returned to normal within two days in dogs with thoracic duct intact and eight days in those with ligated thoracic ducts. The conclusions drawn were that protein formed in the liver entered the blood stream by way of the lymph and that by eight days anastomotic channels were opened up.

Experiments with thoracic duct fistulae in animals, however, do not support this concept There is no doubt that both in man and animals the loss of protein through a thoracic duct fistula leads to considerable plasma protein depletion; but the evidence shows that the depletion of protein in the blood is not nearly as great as the actual loss of protein in the fistula. With the use of polyethylene tubing, lymph may flow from a thoracic duct fistula in an animal for several days. Glenn *et al.* (1949) observed the protein in serum and lymph in six dogs, each with a thoracic duct fistula The right lymph duct had been previously ligated, so that lymph from the thoracic duct was prevented from passing along anastomotic channels to enter the blood stream on the right side. The results of these experiments are given in Table 22. With the constant loss of protein from the thoracic duct, the plasma protein level fell on an average from 6·1 to 3·8 g per cent in 3 to 8 days. In dogs at least 50 per cent of the blood plasma protein is lost per day from the thoracic duct so that in these experiments newly-formed protein must have entered the blood stream. In acute experiments with cats, Korner, Morris and Courtice (1954) also showed that appreciable amounts of

TABLE 21

The protein in plasma, hepatic and intestinal lymph of the dog and cat, g %.

	Number of animals	*Lymph flow ml./kg./hr.*	*Total protein*	*Albumin*	*Globulin*
Dog (Nix *et al*, 1951)					
(i) Plasma	13	—	5·67	3·41	2·26
Hepatic lymph	—	1·0	4·39	2·74	1·65
(ii) Plasma	10	—	5·67	3·47	2·20
Intestinal lymph	—	0·8	2·79	1·90	0·89
Cat (Morris, unpublished data)					
Plasma	5	—	6·60	3·11	3·49
Hepatic lymph	—	0·7	6·12	2·92	3·20
Intestinal lymph	—	1·2	5·26	2·62	2·64

of the liver sinusoids to protein. That most of the protein in the liver lymph comes from the plasma has been shown by labelling the intravascular plasma proteins with the dye, T1824. In such experiments the specific activity of the hepatic lymph approaches that of the plasma much more rapidly than in the case of the intestinal lymph, Fig. 54. If the inferior vena cava just above the liver is constricted to raise the pressure in the sinusoids somewhat, the specific activity of the hepatic lymph may reach that of the plasma within half an hour (Morris, 1956*a*).

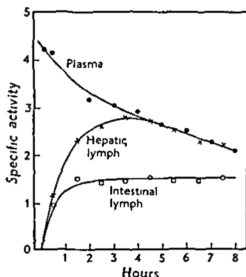


FIG 54.—The specific activity of the plasma and the hepatic and intestinal lymph of the cat following the intravenous injection of the dye T1824 which acts as a label for the plasma proteins

$$\text{Specific activity} = \frac{\text{T1824 concentration mg/litre}}{\text{Total protein g \%}}$$

(By courtesy of Mr Bede Morris)

In addition to the protein which escapes from the plasma, however, it is possible that hepatic lymph contains protein that is manufactured in the liver cells. It is now generally agreed that plasma proteins are readily formed in the liver and that the liver is the most active tissue in the formation of all plasma protein with the exception of gamma-globulin (cf. Madden and Whipple, 1940; Whipple, 1942; Shemin and Rittenberg, 1944; Sprinson and Rittenberg, 1949; Tarver and Reinhardt, 1947; Miller, Bly,

this loss continued for 3 to 4 days, it is apparent that new protein must have entered the circulation.

These experiments on rats give conclusive evidence that newly-formed protein enters the blood stream other than by the thoracic duct. It is possible that some liver lymph passed by the accessory lymphatic channels described earlier. It seems hardly likely, however, that such large quantities of plasma proteins would enter the blood stream by these routes. It would mean that these accessory liver lymph channels convey per day to the blood stream protein and fluid equal to or greater than the total amount present in the plasma initially; that is, the flow from the accessory channels would have to be very much greater than from the main channel and nearly as much as from the entire thoracic duct The balance of the evidence, therefore, suggests that in the liver newly-formed plasma protein may enter the blood stream directly through the walls of the sinusoids

Not only do plasma proteins pass through the walls of the liver sinusoids more readily than through the vascular endothelium of any other region so far investigated, but also, as we have seen in Chapter 2, fat which is present in the blood stream as chylomicrons. The experiments already described suggest that the chylomicrons themselves rapidly penetrate the sinusoidal membrane without first being broken down to smaller complexes.

Lymph flow in liver damage

It seems clear that the lymph from the liver and the intestines normally returns to the blood stream a large amount of extravascular fluid and protein Much consideration has been given to the problem of the origin of ascitic fluid in ascites (*q v*) The liver lymph flow following liver damage is therefore of great importance in the study of this problem.

One of the chief ways of producing ascites in animals is by gradually constricting the inferior vena cava above the diaphragm by means of cellophane bands In time the fibrosis produced constricts the vein, and gross ascites may result. In these circumstances the lymphatic vessels are dilated, the lymph flow and the protein turnover in the liver is very greatly increased (Nix *et al*, 1951) Table 24 (overleaf) shows the results of experiments in dogs. As in any tissue with gross venous congestion, the lymph flow and protein are greatly increased, indicating increased filtration through the sinusoidal membranes when the filtering pressure is raised The spillover of lymph from the liver is thought to be the main source of ascitic fluid in these experiments.

Liver lymph in experimental cirrhosis has also been investigated. In rats and in dogs liver damage resulting in nodular cirrhosis and ascites

TABLE 22

Protein concentration in plasma and in thoracic duct lymph in dogs with thoracic duct fistulae.

Duration of fistula days	*Plasma proteins g %* *Initial*	*Final*	*Lymph proteins g %* *Initial*	*Final*
4	6·46	4·18	3·41	1·87
5	5·43	3·86	3·91	0·94
5	7·08	4·22	4·35	1·92
8	5·81	2·71	4·10	0·34
3	6·05	3·71	3·94	1·06
3	5·83	4·14	2·30	1·09
Mean	6·11	3·80	3·66	1·20

Compiled from data of Glenn, Cresson, Bauer, Goldstein, Hoffman and Healy (1949)

newly-formed protein were added to the circulating blood when the *thoracic duct lymph was drained away from the body.*

Many experiments in rats with thoracic duct fistulae show conclusively that the loss of protein from the thoracic duct is much greater than the loss of protein from the blood (cf. Shrewsbury and Reinhardt, 1952; Forker *et al.*, 1952; Simmonds, unpublished data). The results of Shrewsbury and Reinhardt are summarized in Table 23. The loss of

TABLE 23

Figures for a 4-day period in rats with free access to food and either 1% NaCl or water

	1% NaCl	*Water*
Number of experiments	8	6
Initial body weight g	199	235
Final body weight g.	145	188
Fluid intake ml.	686	108
Lymph output ml	557	59
Urine output ml.	69	36
Lymph protein g.	3·2	1·9
Lymph protein g. %	0·6	3·4
Plasma protein initial g %	5·42	5·59
Plasma protein final g %	3·29	5·21
Total circulating plasma protein initial g.	0·40	0·42
Total circulating plasma protein final g.	0·25	0·39

From Shrewsbury and Reinhardt (1952)

protein in the lymph in 4 days was 5 to 8 times the initial total intravascular plasma protein while the loss of protein from the plasma was only 7 to 40 per cent of the initial amount. In similar experiments Simmonds showed that in rats with an initial total intravascular plasma protein of 0·52 g. the average daily output of protein was 0·46 g. As

this loss continued for 3 to 4 days, it is apparent that new protein must have entered the circulation.

These experiments on rats give conclusive evidence that newly-formed protein enters the blood stream other than by the thoracic duct. It is possible that some liver lymph passed by the accessory lymphatic channels described earlier. It seems hardly likely, however, that such large quantities of plasma proteins would enter the blood stream by these routes. It would mean that these accessory liver lymph channels convey per day to the blood stream protein and fluid equal to or greater than the total amount present in the plasma initially; that is, the flow from the accessory channels would have to be very much greater than from the main channel and nearly as much as from the entire thoracic duct. The balance of the evidence, therefore, suggests that in the liver newly-formed [illegible] the walls of [illegible]

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Lymph flow in liver damage

It seems clear that the lymph from the liver and the intestines normally returns to the blood stream a large amount of extravascular fluid and protein. Much consideration has been given to the problem of the origin of ascitic fluid in ascites (*q v.*). The liver lymph flow following liver damage is therefore of great importance in the study of this problem

One of the chief ways of producing ascites in animals is by gradually constricting the inferior vena cava above the diaphragm by means of cellophane bands In time the fibrosis produced constricts the vein, and gross ascites may result. In these circumstances the lymphatic vessels are dilated, the lymph flow and the protein turnover in the liver is very greatly increased (Nix *et al.*, 1951). Table 24 (overleaf) shows the results of experiments in dogs. As in any tissue with gross venous congestion, the lymph flow and protein are greatly increased, indicating increased filtration through the sinusoidal membranes when the filtering pressure is raised The spillover of lymph from the liver is thought to be the main source of ascitic fluid in these experiments.

Liver lymph in experimental cirrhosis has also been investigated. In rats and in dogs liver damage resulting in nodular cirrhosis and ascites

TABLE 24

Flow and composition of hepatic lymph in normal dogs and in dogs with liver congestion caused by constriction of the inferior vena cava above the diaphragm.

		Normal dogs Mean of 13	*Dogs with hepatic congestion Dog 1*	*Dog 2*
Lymph flow ml /hr		14·8	30	50
Total protein g %	Plasma	5·67	6·05	5·95
	Lymph	4·39	4·41	5·40
Lymph protein mg /hr		662	1,320	2,600

From Nix, Mann, Bollman, Grindlay and Flock (1951)

was produced by the inhalation of carbon tetrachloride for six hours three times a week for 5 to 6 months (Nix, Flock and Bollman, 1951; Nix, Mann, Bollman, Grindlay and Flock, 1951). The results in Table 25 show that in the cirrhotic liver the lymph flow and protein turnover are both greatly increased The mechanisms concerned in the

TABLE 25

The flow and composition of lymph in rats and dogs with cirrhosis of the liver caused by repeated inhalations of carbon tetrachloride The lymph was collected from the cisterna chyli in the rat and from the hepatic duct in the dog.

		Lymph flow ml /hr	*Protein g %* *Plasma*	*Lymph*	*Lymph protein mg /hr*
Rats					
Normal	(10)	0·42 ± 0·06	5·82 ± 0·2	3·06 ± 0·2	12·7 ± 2·0
Cirrhotic	(4)	2·60 ± 0·42	5·16 ± 0·7	4·01 ± 0·3	105·0 ± 4·0
Dogs					
Normal	(13)	14·8	5·67	4·39	662
Cirrhotic	(1)	70	6·45	5·05	3,546

From Nix, Flock and Bollman (1951)

production of ascites will be considered in more detail later in this chapter and in Chapter 8.

GALL-BLADDER

Three lymphatic plexuses may be identified in the wall of the gall-bladder. In the subepithelial layer is a plexus devoid of valves; this plexus does not extend into the stroma of the mucous folds, but it anastomoses by means of vessels which penetrate the muscular layer perpendicularly with a second plexus in the perimuscular layer. In this region the lymphatic vessels are larger, irregular in contour, possess few valves and they drain through short trunks into the third or subserous plexus which is present over the free portion of the gall-bladder. These

subserous vessels are large, rather straight and possess numerous valves. The final channels lie close to the cystic duct and drain into lymph nodes at the head of the pancreas and close to the duodenum (cf. Sudler, 1901; Winkenwerder, 1927).

Lymph may be collected by cannulating one of these final collecting ducts McCarrell, Thayer and Drinker (1941) found that the flow in the cat was large, varying from 11·2 to 90 7 mg./min, i e. 0 7 to 5·4 ml./hr. This high rate of lymph flow was no doubt due to connections with the lymphatics of the liver. Such communication has been generally recognized (cf. Sappey, 1874; Sudler, 1901; and Baum, 1918) although Winkenwerder could not demonstrate connexions between the two systems by retrograde injections of the gall-bladder and liver lymphatics of the cat. McCarrell *et al* injected graphite and the dye T1824 into the liver and found that the injected material rapidly entered the gall-bladder lymphatics

Not only is the lymph flow considerable, but the protein concentration is also high, the same as in liver lymph. In a series of cats McCarrell *et al.* found that on an average the protein content of the plasma was 5 74 per cent, of liver lymph 5·58 per cent and of gall-bladder lymph 5 61 per cent It would seem, therefore, that most of the lymph collected from the main lymphatic vessels of the gall-bladder comes from the liver. Because of this communication there is no information regarding the composition and flow of lymph actually produced in the gall-bladder.

Skin and Subcutaneous Tissues

Anatomical distribution of lymphatics

In the development of the skin lymphatics, a single primary plexus of unvalved vessels covering the whole body surface is first formed along the inner border of the corium as outgrowths of the jugular and iliac lymph sacs (Sabin, 1904) From this primary plexus, which later becomes valved, sprouts grow outwards into the corium to form the superficial plexus, extending into the papillae In general, in the fully developed organism, the lymphatics of the skin have been observed to consist of a superficial plexus in the corium and another plexus lying in the deeper part of the corium or in the adjacent subcutaneous tissue (Sappey, 1874). No lymphatic vessels have been demonstrated in the epidermis. Fig. 55 (Kampmeier, 1928*a*) is a reconstruction of the two plexuses There is depicted a superficial network of vessels with irregular meshes and containing few valves, from which branches here and there drain into the more slender valved vessels lying deeper Forbes (1937–1938) has

modified this distribution slightly by describing in human skin two superficial plexuses without valves, lying in the outer two-thirds of the corium and the deeper valved plexus occupying the inner layer of the dermis and the superficial zone of the subcutaneous tissue This pattern,

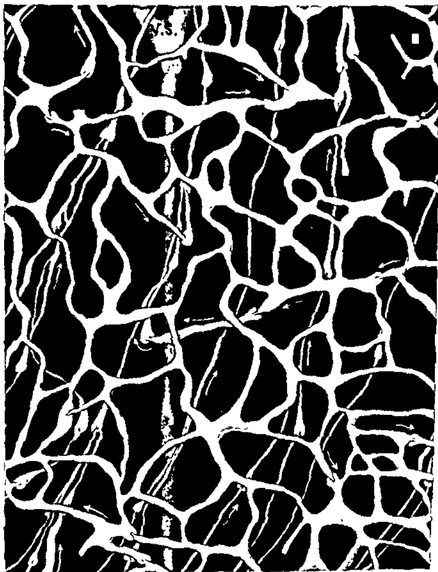


FIG 55 —Reconstruction of a small portion of the lymphatic plexus in a cutaneous and subcutaneous area of the leg of a 130 mm (4 3 months) foetus

demonstrated histologically, was found to be much the same in all areas; in the sole of the foot and the palm of the hand, however, there is a richer network than elsewhere.

In the living animal the lymphatics of the skin have been displayed by the intracutaneous injection of dyes or graphite. Hudack and McMaster (1932) injected a small amount of dye into the skin on the outer side of the ear of the mouse. The dye readily passed into the lymphatic capillaries, and by varying the depth at which the dye was placed, they revealed a superficial and a deep plexus of lymphatics, intercommunicating and both possessing valves. In appearance the lymphatics were irregular channels of highly varying size and shape, the smallest lymphatics observed by the method being 15 μ in diameter, much larger than the blood capillaries. No matter where dye was injected in the ear, it was always transported towards the head; it could only be forced to the periphery by rupturing valves. In man the superficial plexus was seen to lie in the papillary stratum of the corium with blind ends extending into the papillae, while deeper in the skin, in the lower layer of the corium and in the subcutaneous tissue, another plexus of lymphatic capillaries was observed (Hudack and McMaster, 1933). So close-meshed were these lymphatic networks that it was not possible to make an intradermal injection without injecting the lymphatics. In practice, therefore, every intradermal injection is an intralymphatic injection as well.

Flow and composition of lymph from the skin

The skin is a very extensive organ with a high extracellular fluid phase and with dense networks of lymphatic capillaries. Under normal circumstances, however, the flow of lymph from the skin is relatively small. If a cannula is inserted into a lymph trunk draining the foreleg of a dog, for example, there is usually no flow of lymph when the leg is at rest; but any movement, either passive or active, or massage along the course of the duct will cause lymph to flow into the cannula. This lymph has a lower protein concentration than has lymph from most other organs of the body; in animals it usually lies within the limits of 0.5 to 2 per cent. Although at rest the lymph flow from the skin is not high, and the protein concentration is relatively low, the lymphatics are essential for the removal of this protein. If they are blocked lymphoedema and elephantiasis are observed. A more detailed account of flow of lymph from the skin in normal circumstances, however, will be given in Chapter 4.

The lymphatics of the skin play a prominent rôle in many pathological conditions. A large volume of extracellular fluid, for example, may accumulate in the skin giving rise to visible oedema, the protein

modified this distribution slightly by describing in human skin two superficial plexuses without valves, lying in the outer two-thirds of the corium and the deeper valved plexus occupying the inner layer of the dermis and the superficial zone of the subcutaneous tissue. This pattern,

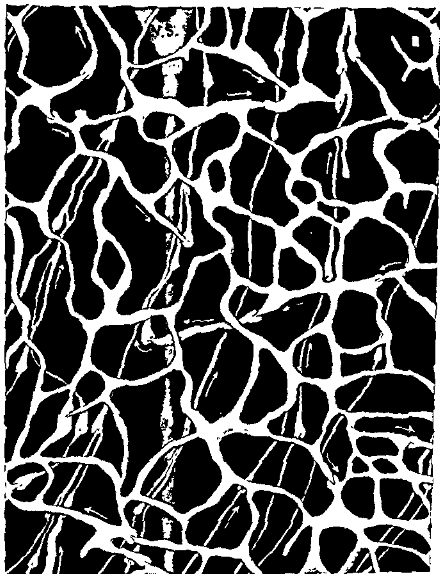


FIG 55.—*Reconstruction of a small portion of the lymphatic plexus in a cutaneous and subcutaneous area of the leg of a 130 mm (4.3 months) foetus.*

White rectangle in the upper corner indicates natural size of the area shown. Note the numerous valves, and the drainage of the subcutaneous plexus into the deeper, more regularly disposed, and more slender lymph channels. V.S., vena saphena magna

(From Kampmeier, 1928, Fig. 31, p. 442)

tion of particles. Boyland *et al.* found that the greater the air flow, the more the larger particles were held up in the nose, while Davies showed that slow deep breathing enhanced the retention of small particles on the surfaces of the bronchioles and alveoli. This is readily understood when we realize that the deposition of the larger particles is by the phenomenon of impingement, whereas that of the smaller particles is by sedimentation.

Not only particles, but vapours, too, may be differentially held up in the respiratory tree. Cameron, Gaddum and Short (1946) showed that whereas the vapour of mustard-gas (dichlorodiethylsulphide) and of nitrogen-mustard (β,β^1-dichlorodiethyl methylamine) was nearly all held up in the nose, phosgene (carbonyl chloride) largely passed through the nasal cavity and affected the lower respiratory tract.

Having been deposited on the walls of the respiratory tract, these droplets or particles may be dealt with in different ways and the part played by the lymphatics will vary with the circumstances. The particles may be soluble, in which case the smaller molecules may be absorbed; they may be irritant in which case the secretion of mucus will be increased but at the same time the membrane may be damaged; they may be living organisms, as in the case of viruses or bacteria, and so may multiply in the membrane.

The rôle of the lymphatics in removing the extracellular fluid and protein and in absorption from the outside air will be considered firstly in the upper respiratory tract where the air is largely filtered, moistened and warmed, and then in the lungs where the gaseous exchange occurs.

The Upper Respiratory Tract

(i) *The nasopharynx.* Anatomically, the nasal mucous membrane differs in different regions. The respiratory part of the membrane is columnar and ciliated and in man these cilia direct the entire mucous drainage toward the nasopharynx (Lucas and Douglas, 1934) In the olfactory region, however, which consists of the superior nasal concha and the opposed part of the septum, the mucous membrane consists of the bipolar olfactory cells and supporting epithelial cells which are not ciliated. Much attention has been focussed on absorption through the olfactory membrane because of the possible communications by way of the olfactory nerve fibres, with the central nervous system It has been thought that some viruses may gain entry to the body in this way, but this will be considered in more detail later.

Beneath the epithelium in the submucosa is a very rich lymphatic plexus, Figs. 56 and 57 (cf Yoffey and Drinker, 1939–1940; Yoffey, 1949). The lymph from these vessels drains into the deep cervical lymphatics, which after passing through the deep cervical nodes enter

concentration of this fluid may vary from very little as in nephrosis to the level in the plasma as in thermal burns. In all these conditions the lymph vessels play a part in restoring the fluid balance to normal. In many other circumstances such as infection, injury and inoculations the lymphatics are involved, further discussion of these topics will be found in Chapter 8

THE RESPIRATORY TRACT

We are concerned here with the passage of plasma protein through the capillaries of the systemic circulation in the upper respiratory tract and through the capillaries of the low pressure lesser circulation in the alveoli. In addition there is always the possibility of absorption of substances from the outside air into the lymph vessels. The surfaces of the respiratory tract are continually in contact with the atmospheric air which often contains particles of different sizes, both inanimate and living These particles may be arrested in one or other part of the respiratory tree where various lines of defence must be overcome before the substance can gain entrance to the lymphatics (Fenton and Larsell, 1937) In the upper respiratory tract, which is, in the main, lined by columnar ciliated epithelium, the first barrier is a layer of mucus and the action of the cilia ; the second is the epithelium itself. In the alveoli these barriers are not present—the existence even of an attenuated epithelium has been hotly debated—but here phagocytosis plays an important rôle in preventing the entrance of particles into the body (cf. Robertson, 1941). Particles or other substances which do penetrate these barriers come into close association with the lymphatic capillaries forming the next line of defence.

The site of deposition of inhaled particles in the respiratory tree depends to a large extent on their size. Boyland, Gaddum and McDonald (1947) showed that in man and in various experimental animals the larger the particle the more it is held up by the nose Practically all particles larger than 15 μ in diameter were deposited on the nasal mucous membrane, whereas those of the order of 1 μ in diameter passed through the nose. Davies (1946, 1949) concluded that nearly all droplet particles greater than 12 μ in diameter were retained in the nose, while those of 4 μ diameter were deposited mainly in the nose, bronchi and bronchioles Smaller particles, however, from 0·8–1·6 μ in diameter were deposited mainly in the alveoli and fine bronchioles, whereas those of 0·2–0·3 μ in diameter were in part retained in the alveoli but in the main were breathed out again. Yet smaller particles deposited in the alveoli and small bronchioles because of their Brownian motion.

The type of breathing was also found to affect the degree of deposi-

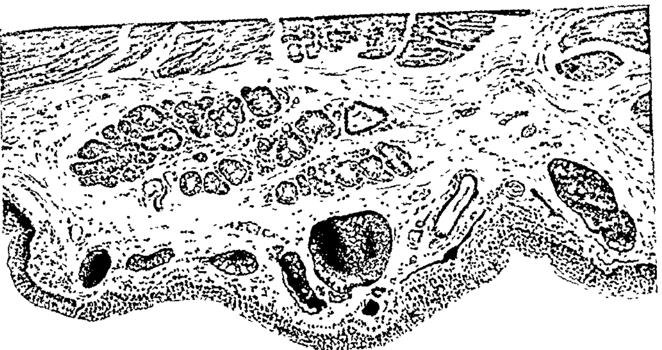


FIG 57.—Histological section of the nasopharyngeal mucosa of the monkey

The lymphatics in the tunica propria are injected with a dilute solution of India ink. Note their number, their large size and their proximity to the surface. (From Yoffey and Drinker, 1940, Fig. 3)

the veins at the base of the neck with, or close by the entrance of, the thoracic duct on the left side and of the right lymph duct on the right side (cf Blumgart, 1924, Larsell and Fenton, 1936; Yoffey and Drinker, 1938, 1939–1940; Yoffey, 1941). In the cat and dog the deep cervical nodes consist of one large gland on either side close to the bifurcation of the common carotid artery, while in the rabbit there are frequently two nodes and in the monkey a chain of five or more on each side.

The rich lymphatic plexus in the nasal mucosa is no doubt necessary to ensure the removal of tissue fluid and so to provide a clear airway This membrane is also very vascular; great changes in blood flow may be brought about by variations in the air breathed and by nasal irritants, leading to changes in the amount of tissue fluid formed. Not only may the vascularity be altered, but the permeability of the capillary membrane



FIG. 56 —One of the turbinate bones showing the lymphatics of the nose

The smaller vessels are completely filled with ink, whereas in the larger vessels the lumen is almost empty, and the ink has adhered to the walls

(From Yoffey, 1949)

to proteins may be increased by many irritants, resulting in oedema. The rôle of the lymphatics in removing oedema fluid has been observed experimentally by cannulating the deep cervical duct and collecting lymph before and after the application of an irritant to the nose (Cameron, Courtice and Short, 1947) When the arsenical vesicant, lewisite, was applied to the nasal mucous membrane, the resulting oedema greatly increased the tissue tension, so that the lymph flow often became spontaneous without passive movement of the head. The protein content of the lymph rose until it reached the level in the plasma, Fig. 58 In any nasal infection or irritation the lymph flow will no doubt be increased by the increased tissue tension produced and by the pulsation of the dilated blood vessels (Robison, 1950) The close approximation of the mucous membrane to the rigid bony surfaces of the nose ensures a steep rise in tissue pressure as soon as excess tissue fluid is formed, and it is

normal physiological saline (McCarrell, 1939) solutions of prussian blue (Blumgart, 1924; Clark, 1929; Rake, 1937), phenolsulphonphthalein and potassium iodide (Blumgart, 1924), pituitrin (Blumgart, 1922), lead carbonate (Blumgart, 1923) and mecholyl (van Dellen, Bruger and Wright, 1937) have been found to penetrate the normal nasal mucosa.

The rapid absorption of water through the nasal mucosa was shown by McCarrell, who cannulated the cervical ducts in cats and collected the lymph before and during the irrigation of the nose with distilled water. The lymph flow rapidly increased and the protein level in the lymph fell, indicating that some of the water, after penetrating the mucosa, entered the lymphatic vessels. The absorption of isotonic saline was very much slower and was scarcely detectable by this method The uptake from the nose of other small molecules into the body, presumably by both the blood and lymphatic vessels, has been ascertained in various ways. Phenolsulphonphthalein and potassium iodide were observed in the urine; pituitrin prevented polyuria in patients with diabetes insipidus and mecholyl was shown to cause a fall in blood pressure. The exact pathway of entrance of these substances was not ascertained, but it would seem that they penetrated the mucosa by diffusion. With prussian blue, however, the pathway could be followed, for, on acidifying the tissue post mortem, the prussian blue showed up as blue granules It was found by Blumgart in the cat, by Clark in the rabbit and by Rake in mice that prussian blue penetrated the olfactory region of the mucosa The blue granules were observed both in and between the olfactory cells, in the loose connective tissue of the submucosa and in the lumen of some of the thin-walled lymphatic vessels and in the blood vessels. These experiments suggest that the small crystalloids enter the body by diffusion mainly through the olfactory region of the mucosa.

Colloids. The diffusion rate of colloids is very much less than that of the smaller crystalloids, so we would expect them to enter the lymphatics with much less ease Yoffey and Drinker (1938) studied the absorption of Trypan blue and of Evans blue (T1824), molecules with a molecular weight of about 1,000, when applied to the normal nasal mucosa of animals. By cannulating the cervical lymph ducts they found that these dyes appeared in the lymph in 15-30 minutes in the cat and monkey, 51-53 minutes in the dog and 14 minutes in the rabbit Although these molecules penetrated the nasal mucosa and stained the lymph blue, the amount of absorption was probably small.

Using larger molecules, Yoffey, Sullivan and Drinker (1938) found that egg albumin passed into the lymph; serum albumin did not appear in the lymph of the cat but it did in the rabbit, while horse serum was not detected in any animal Nor did the dye T1824 appear in the lymph when it was added to horse serum to form a dye-protein complex. It

this rise in tissue pressure that increases the lymph flow and so clears the mucous membrane of the excess fluid and extravascular protein. Besides fluid which filters through the capillaries of the nasal mucosa, some cerebrospinal fluid also passes into the nasal mucosa to be absorbed in part at least by the nasal lymphatics.

Although the most prominent function of the lymphatics of the nasal mucosa is to remove any excess extravascular fluid and protein, as in any other region of the body, attention has been focussed on the absorption of substances and particles which have been deposited on the nasal mucosa from the outside air. This is especially the case regarding the

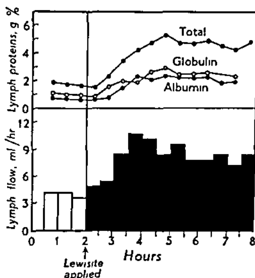


FIG 58—The flow and protein composition of lymph from the cervical duct of the dog, before and after the application of the arsenical vesicant, lewisite, to the nasal mucosa

(From Cameron, Courtice and Short, 1947)

portal of entry into the body of certain viruses and bacteria. Viruses, themselves, are very small particles varying in diameter from 0·01–0·015 μ in the case of poliomyelitis virus to 0·25–0·275 μ in the case of psittacosis virus (Mueller, 1941) As such they would not to any extent be deposited in the nose; but they are generally inhaled in droplets which have a very much larger size, so ensuring that they will be deposited largely on the nasal mucosa.

In an endeavour to ascertain the pathways of entrance into the body of these particles, substances of varying molecular or particle size have been applied to the nasal mucosa and their penetration of the membrane studied.

Water, crystalloids and other small molecules. Besides water and

When poliomyelitis virus, which is only 0·010–0·015 μ in diameter, was applied to the nose, however, Yoffey and Drinker (1939) could not detect it in the cervical lymph of monkeys even though these animals later developed paralysis. They collected lymph at several different stages from the first few hours after instillation up to the development of paralysis. From these experiments they concluded that the strain of virus used did not spread by way of the lymphatic vessels and nodes, or, if it did, it was present in concentrations below the minimal infective dose.

So far as viruses are concerned, it is fair to conclude that penetration of the nasal mucous membrane cannot be predicted on the basis of virus size, and that only viruses which multiply in the membrane enter the lymph in amounts great enough for detection.

Bacteria If size alone were the only significant factor, bacteria, like inanimate particles, would not penetrate the mucosa and enter the lymphatics Blumgart (1924), using a fixed suspension of staphylococcus aureus, found that these organisms were not absorbed through the nasal mucosa. Living organisms, however, such as meningococcus and haemophilus influenzae, apparently multiply on and within the nasal mucosa, from which they spread to other parts of the body. Schulz, Warren and Drinker (1938) using rabbits anaesthetized with nembutal, instilled rabbit-virulent type III pneumococci into the nose, the trachea being cannulated to preclude lung absorption The organisms were frequently collected from the cervical lymph at the end of one hour, and rarely failed to be present in four hours. The pneumococci also entered the blood, but invariably were found first in the lymph. The organism employed was not ingested by phagocytes in the rabbit and apparently multiplied successfully and penetrated the nasal membrane only after damaging it. Rake (1937), investigating the possible pathway from the nose through the cribriform plate to the brain, in mice, instilled cultures of virulent type III pneumococci and of salmonella enteriditis into the nasal cavity. He found in as short a time as 1 to 30 minutes that the olfactory area of the brain gave in most cases a positive culture of these organisms. He concluded that these organisms could rapidly penetrate the olfactory mucosa by passing between the olfactory cells.

(ii) *The Accessory Sinuses* The paranasal sinuses—frontal, ethmoidal, sphenoidal and maxillary—are all lined with ciliated columnar epithelium which is continuous through openings with the mucosa of the nasopharynx Absorption of substances through the normal mucosa is probably very slow. McCarrell (1940) injected a solution of T1824 into the frontal sinuses of the dog, but in 4 hours obtained no dye from the cannulated cervical lymphatics. When substances are actually injected into the mucosa, however, they are readily transferred by the lymphatic

would seem, therefore, that molecules up to the size of those of egg albumin will normally penetrate the nasal mucosa, whereas larger ones such as serum proteins are excluded.

Inanimate particles. Blumgart (1923) insufflated lead carbonate into the noses of dogs and cats, in which he had previously cannulated the trachea and tied the oesophagus so as to preclude the possibility of absorption from the lungs and digestive tract. Free particles of lead carbonate deposited on the mucous membrane were dissolved very slowly, if at all. It is well known that such particles are dissolved after phagocytosis, and Blumgart's finding of considerable lead absorption after intranasal instillation of lead carbonate would seem to indicate penetration of the mucosa by lead carried within the numerous phagocytes present on the surface of this membrane. On the other hand, powdered ivory black consisting of particles as small as 2 μ in diameter did not penetrate the mucosa. Yoffey and Drinker (1938) instilled a suspension of hydrokollag (carbon particles) into the nose of the cat but none of the particles appeared in the cervical lymph Inert particles, therefore, unless they are soluble, do not penetrate the nasal mucosa.

Viruses. All viruses are much larger than the serum protein molecules and would not therefore be expected to penetrate the normal mucous membrane of the nose unless they damaged the epithelium Yoffey and Sullivan (1939) instilled vaccinia virus into the noses of the monkey, cat and rabbit and collected the lymph from the deep cervical ducts. In the first twelve hours after instillation they did not find any of the virus in the lymph. This indicates that vaccinia virus which has a diameter of 0·125–0 175 μ (1250–1750 Å, compared with serum proteins of 30–40 Å) does not readily penetrate the nasal mucosa. In the rabbit, which is susceptible to vaccinia, the virus becomes established in and on the nasal mucosa and then at periods from 12 hours up to 7 days (or longer) the virus was consistently found in the cervical lymph in association with the lymphocytes.

These experiments indicate that although a virus may be too large to gain rapid entrance into the body through the nasal mucosa, it may establish itself and later enter the lymphatics to be disseminated by the lymphocytes throughout the body. Having escaped from the blood vessels the lymphocytes will re-enter the lymphatics and so may distribute the virus to all the lymph nodes of the body. It seems that in man some of the viruses, such as those of measles, chicken-pox, small-pox and the common cold, gain entrance to the body in this way If this is so, the lymphatic system plays an important part in the general dissemination of the virus (cf Yoffey, 1949). It is probable, however, that other viruses such as the virus of serum hepatitis may be present in the plasma other than in association with the lymphocytes.

surface and embedded in the bronchial mucous membrane. By analogy with the situation in the alveoli, one would expect such phagocytes to migrate along lymphatic routes, and to come to rest in lymph nodes, principally at the root of the lung. The great majority of particles that adhere to the surface of the bronchial mucosa, however, are eliminated from the lung by ciliary action and by coughing (cf. Robertson, 1941)

Pulmonary Alveoli

Lymphatic pathways

The lungs are provided with an abundant supply of lymphatics, a superficial network in the pleura pulmonalis and deeper vessels which accompany the bronchi, pulmonary artery and its ramifications and the pulmonary veins, Fig. 59 These two sets are in communication with each other in the pleura and at the hilum of the lung (Miller, 1937) The lymphatics of the pulmonary pleura will be dealt with in a subsequent section. The respiratory portion of the lung is drained by the deep vessels which present certain anatomical features of interest physiologically. One such feature is the absence of lymphatics in the walls of the atria and alveoli. The bronchial tree and the pulmonary arterial tree are enveloped by extensive intercommunicating plexuses up to the distal ends of the alveolar duct. At these points, the lymphatics communicate freely with the plexuses surrounding pulmonary venous radicles, but extend no further along the respiratory tree Another feature of physiological interest is the scarcity of valves in the deep lymphatics except where the pulmonary venous lymphatics join the pleural set, and where the deep lymphatics converge on the hilar nodes In the hilar region the valves point towards the nodes.

Lymph from the lungs may flow in two directions. Some, probably from the periphery of the lung only, flows outwards into the pulmonary pleural lymphatics, as shown by small deposits of carbon in these vessels after exposure to carbon dusts Most of the lymph, however, seems to flow inwards towards the hilum. The onward movement of lymph into the hilar nodes must depend on a gradient of pulmonary pressure together with the centrally pointing valves in the hilar region There is no evidence for specialized absorptive areas as in the serous cavities The lymphatic drainage seems to be equally rich in all parts of the lung and Miller was unable to confirm suggestions that lymph circulation was defective at the apex of the lung

Until fairly recently little was known of the volume or composition of the lymph from the lungs Although anatomists had earlier shown that the lymphatics of the lung drain to the right lymph duct in man (cf. Sappey, 1874) it was not until 1942 that Warren and Drinker demonstrated that lymph from both right and left lungs in dogs could

vessels to the retropharyngeal and deep cervical nodes. Larsell and Fenton (1936) injected prussian blue, trypan blue and potassium ferrocyanide into the frontal sinuses of the cat; Larsell, Veazie and Fenton (1938) injected cultures of streptococci into the frontal sinuses of the cat and maxillary sinuses of the rabbit; Mullin (1926) injected the mucosa of these same sinuses with India ink and tubercle bacilli, while Dixon and Hoerr (1944) injected India ink into the mucous membrane of the maxillary sinus of the rabbit. In all cases the lymphatic pathway was the same When fairly large amounts were used the substances injected could be found in the lungs, liver and spleen. Some of the bacteria may, therefore, pass through the cervical lymph nodes to enter the blood stream and so reach the lungs, and later other organs such as the liver and spleen.

The method of spread of infection from these sinuses to the lungs is probably by one of two routes—direct spread by inhalation or by the lymphatics and then the blood stream.

Although the lymph flow from the normal sinus mucosa is probably small, as soon as the membrane becomes oedematous and congested from inflammatory processes, the lymph vessels dilate and the flow increases. Because the membrane is tightly attached to the rigid bone the tissue tension rises rapidly in inflammation as in the nose.

(iii) *Larynx, Trachea and Bronchi.* It is generally believed that direct absorption through the mucous membrane lining the larynx, trachea and bronchi is slight. The lymphatic supply—as in the nasopharynx and in the lung—is large, one network of capillary lymphatics being in the mucosa, and another in the submucous tissue. This prolific supply of absorbing vessels may perhaps best be related to the fact that the whole upper respiratory tract is so often subjected to inflammation, both from disease and from irritants of various sorts While lymph movement in the tracheal lymphatics is slight under perfectly normal conditions, the mechanism for lymph transport is there, and can be utilized whenever transudation and exudation from blood vessels require it.

Drinker and his colleagues (Drinker and Yoffey, 1941) flooded the mucous membrane of the trachea with the dye T1824 which readily penetrates the nasopharyngeal mucosa At autopsy five hours later several fine lymphatics on the outer surface of the trachea were seen to be stained blue, and the colour had reached several of the small nodes along the trachea. The amount of dye absorbed, however, was very small

That particulate material on the surface of the mucosa may be carried into the depths of the membrane is shown from experiments by Duthie (1930) who allowed mice to inhale soot for a week. On histological examination, this investigator found phagocytes containing soot on the

The formation and flow of lung lymph in the anaesthetized dog with unopened chest have been studied by collecting lymph from the right lymph duct in various circumstances (Warren, Peterson and Drinker, 1942; Drinker, 1945; Cameron and Courtice, 1946; Cameron, Courtice and Short, 1947; Drinker and Hardenbergh, 1947, 1949). While it is very convenient to collect lung lymph from the right lymph duct without the necessity of applying artificial respiration, it must be remembered that the lymph from the heart and from the pleural and peritoneal cavities

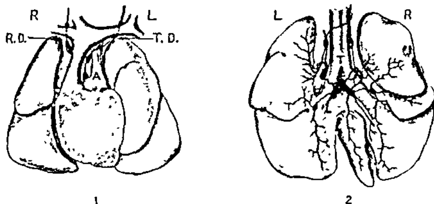


FIG 60—Semidiagrammatic representation of the essential drainage paths for lung lymph in the dog

1 Anterior view The entrance of the right lymph duct into the subclavian vein Cervical lymphatics *R* and *L* above the vein. There is frequently a communicating vessel, not shown in the figure, between the right lymph duct and the thoracic duct *R D*, right duct, *T D*, thoracic duct

2 Posterior view The typical arrangement of the lung lymphatics at the root of the lungs and a short distance above *T*, trachea

(Redrawn from Drinker, 1945)

also largely drains into this duct (Warren and Drinker, 1942; Courtice and Simmonds, 1949*b*; Courtice and Steinbeck, 1950*a*). In some instances, too, there are anastomotic connexions in the thorax between the thoracic and right lymph ducts. The presence of milky or opalescent lymph in the right duct indicates that such connexions are functioning and the lymph is to some extent coming from the intestines. This may occur with normal breathing or may only become apparent when respiratory movement is greatly increased. Combined with these difficulties is the fact that cannulation of the right lymph duct is not an easy procedure, so that information concerning lymph from the lungs can only be gained by patient and somewhat tedious experimentation.

Normal lung lymph

In collecting lymph from a duct draining the lungs in dogs with open chest and positive artificial respiration, Warren and Drinker found that

be collected from a vessel running to a lymph node on the right side of the trachea, between it and the aorta, Fig. 60. They investigated lymph drainage from the various parts of the lung by injecting the dye, T1824, intrabronchially under anaesthesia and noting which lymph nodes

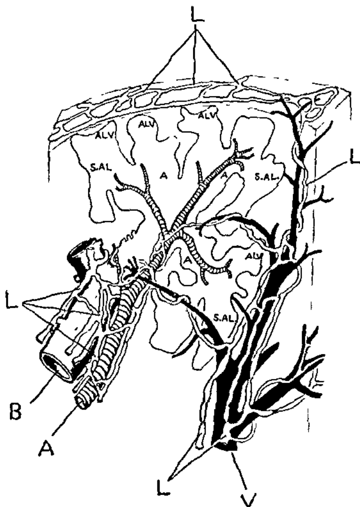


FIG 59—Diagrammatic representation of lymphatics of lung

A, pulmonary artery, *V*, pulmonary vein, *B*, bronchiole, *L*, lymphatics, *a*, atrium *s al*, alveolar sac, *alv*, alveolus

(Redrawn from Miller, 1937)

and efferent vessels subsequently became dyed. They concluded that lymph finally entered the right lymph duct from all parts of the lungs except the upper section of the left lung, which drained into the thoracic duct. In these experiments they cannulated the lung lymphatic in the thoracic cavity, thus necessitating the use of positive artificial respiration.

demonstrated, but Short and Potter and Loosli (1951) described changes in the later embryonic period which resulted in the alveolar wall, at birth, consisting almost entirely of capillaries and of ground substance and reticulum in which they lay. Their accounts differ concerning the way in which this was brought about. The affirmative side of the controversy was taken by Miller (1937) who called attention to a continuous lining of swollen cells seen when oedema of the alveolar walls was present The relevance of this observation has been questioned since insufficient nuclei could be counted in normal alveolar walls to supply the lining seen in pathological conditions Recently, Low (1952, 1953) has demonstrated a continuous cytoplasmic lining, in some places exceedingly thin and in others expanding to enclose an epithelial nucleus, by electron microscopy of rat's lung fixed in 1 per cent osmic acid. Macklin (1954) has postulated that this alveolar membrane demonstrated by Low is coated with a very thin mucoid film. The papers cited should be consulted for details of the controversy and for further references to the literature.

Removal of particles

We have seen that while the larger inhaled particles are held up in the nose and bronchi, the smaller ones may be deposited in the alveoli. The principal means of removal of inhaled dust particles reaching the alveoli is that of phagocytosis by large amoeboid cells, the alveolar phagocytes or "dust cells" (cf. Robertson, 1941; Macklin, 1951) After engulfing particulate material these cells migrate to the cilia bearing areas of the bronchial tree, whence they are transported up the "mucus escalator" by ciliary action to the pharynx and swallowed Some macrophages with engulfed particles, however, slowly penetrate the alveolar wall, especially in the region of the alveolar duct, pass into the tissue fluid and lymphatics and ultimately reach the hilar nodes Nearly all the intracellular particulate material drifting slowly to the hilar nodes appears to be arrested there. If particles such as carbon (Lemon and Higgins, 1932*a*, Carleton, 1933–1934) or silicate of nickel and magnesia (Fang, Field and Drinker, 1933) are introduced into the lung in aqueous suspension, some of the particles after being taken up by the alveolar macrophages may appear in the lymphatics much more quickly—within a few hours—than do inhaled particles. For more detailed information the review by Robertson should be consulted

Recently, interest in the problem has been stimulated by the therapeutic possibility of irradiating regional lymph nodes by radioactive colloids given intratracheally, and by the need for data on the rate and pathways of elimination from the lungs of radioactive dust. Hahn, Meneely and their colleagues have measured radioactivity in hilar lymph

in 18 animals the average lymph flow was 1·1 ml./hr. and the protein content of the lymph was 3·7 per cent They pointed out, however, that this was not the only lymph vessel coming from the lungs; others were ligated. This, therefore, is probably not the total protein and fluid returned by the lung lymphatics. The output from the right lymph duct cannulated at the base of the neck should include nearly all the lung lymph, but also, unfortunately, lymph from other tissues too In a series of 21 anaesthetized dogs breathing normally, Courtice (1951) found that the average lymph flow from the right lymph duct was 2·3 ml./hr. with a protein concentration of 3·7 per cent. In a day this would amount to about 2 grams of protein or 3·6 per cent of the circulating plasma protein. In similar experiments the thoracic duct lymph per day contained 47·5 per cent of the circulating plasma protein. It is impossible to say in these experiments how much of the protein in the lymph from the right lymph duct came from the respiratory portions of the lung and how much from the bronchial mucosa, heart or serous cavities. It is evident, nevertheless, that despite the very large blood flow through the pulmonary circulation, relatively little protein normally escapes from the pulmonary capillaries in anaesthetized animals. Were this protein not efficiently removed, however, protein-rich fluid would soon accumulate in amounts great enough to hinder the proper aeration of the alveoli. Whether this leakage of protein is greater or less in an unanaesthetized animal has not been determined.

The protein concentration in the normal lymph is always fairly high As pointed out in Chapter 2 the protein concentration of lymph is not by itself a guide to the permeability of the capillary membrane. The low flow and high protein concentration of the lymph are probably associated with the low capillary pressure and not with a high permeability of the capillary membrane.

Lymphatic absorption from the alveoli

When tissue fluid is formed in excess, the lymphatics may be called upon to remove extravascular protein, not only from the interstitial tissue but also from the alveoli. Particles from the outside air, too, may be deposited in the alveoli. Before any fluid or particles in the alveoli can reach the lymphatic capillaries, they must pass through the alveolar membrane. The existence of an alveolar membrane in the adult animal has, however, been controversial. In adult animals and man, Loosli (1935) and Short (1950) were unable to detect an epithelium, nucleated or non-nucleated, covering the pulmonary capillaries, despite efforts to make any such lining more apparent by prior collapse of lung lobes or the production of oedema of alveolar walls. The existence of a complete respiratory epithelium during early embryonic life has been repeatedly

Removal of fluid and protein

Since Colin (1873) found that a horse tolerated the intratracheal administration of 21 litres of water in 3½ hours without apparent ill effect, a number of investigators have confirmed the rapid absorption by the lower respiratory tract of protein-free saline or water. Courtice and Phipps (1946) showed that distilled water was absorbed more rapidly than 0·9 per cent NaCl solution, while Courtice and Simmonds (1949*a*) observed that the blue dye T1824 was more rapidly absorbed from the alveoli when it was dissolved in water than in 0·9 per cent NaCl. Qualls, Curtis and Meneely (1953) found that labelled ions (I^{131} or Na^{24}) appeared in the blood stream more rapidly after administration in distilled water than in 0·9 per cent NaCl solution. Freely diffusible solutes appear rapidly in the blood stream after intratracheal administration, and drugs, antibiotics and sulphonamides have been effectively administered by inhalation as aerosols (cf. Mutch and Hoskins, 1944; Mutch and Rewell, 1945; Pringal, 1948). In such circumstances absorption mainly takes place directly into the pulmonary blood capillaries, although some of the substance introduced will also enter the lymphatics.

Protein-rich fluid in the alveoli, on the other hand, despite the profuseness of the pulmonary lymphatics and the continual movement to which they are subjected by expansion and relaxation of the lungs is only slowly removed, much more slowly, for example, than from the serous cavities. Whereas 6 ml./kg. of homologous plasma can be removed by the lymphatics of the rabbit in 5–8 hours from the peritoneal cavity and in 16–24 hours from the pleural cavity, it takes 3–5 days for its removal from the lungs (cf. Courtice, 1949). Fox (1936) concluded, from his own experiments and from a review of the literature, that the lungs were only slightly permeable to antibody protein, homologous or heterologous [illegible]

lymph movement, and lymph was collected from thoracic and right lymph ducts. No dog plasma albumin and only traces of foreign albumin were detectable in right duct lymph, none in thoracic duct lymph or the blood stream. Courtice and Simmonds (1949*a*) confirmed this by observing that absorption of dye-labelled homologous plasma protein from the lungs was negligible in rabbits under nembutal anaesthesia. However, on recovery from anaesthesia, or in rabbits anaesthetized only for intratracheal injection, absorption, though slow, was not inconsiderable and could account for the rate of disappearance of protein-rich pulmonary oedema fluid observed in unanaesthetized animals and man,

nodes after intratracheal or intrabronchial injection in dogs of radioactive colloidal gold and silver (Hahn and Carothers, 1953; Hahn *et al*, 1952; Meneely *et al.*, 1951, 1953). They found that negligible amounts of colloidal gold reached the hilar lymph nodes after injection of radioactive colloidal gold, or passed through them to be trapped in liver and spleen; almost all the colloidal suspension was fixed locally. Colloidal silver, or gold coated with silver passed to the hilar lymph nodes more readily but did not pass further on into the main lymphatic trunks and blood stream. Although quite high concentrations of radioactivity per unit mass of lymphoid tissue were achieved in this way, the percentage of injected dose recoverable in regional lymph nodes was small—about 2 per cent. Particles of plutonium, uranium and their fission products, inhaled as aerosols and deposited in the alveoli, were subsequently removed slowly but steadily, almost entirely by way of the bronchial tree (Scott *et al.*, 1949). There was no evidence of considerable absorption into the lymphatic system. Insoluble particles seemed to be retained on the air side of the alveolar lining and were still being removed via the bronchial tree as late as 8 months after exposure to radioactive dust. The particles used by both these groups of workers were small, average diameter 0 05 μ and less than 1 μ respectively.

The removal of homologous red cells from the lungs has not been studied quantitatively, but histological studies do not suggest that lymphatic absorption is important (Magarey, 1951). Experiments concerning the quantitative lymphatic removal of bacteria from the alveoli are also few in number and were made before it was recognized that the lung lymphatics drain mainly into the right lymph duct. Those inhaled non-pathogenic bacteria which reach the alveoli appear to be quickly taken up by phagocytes and digested. Small numbers of inhaled pathogenic organisms are also no doubt partly dealt with in this way Some, however, rapidly penetrate the alveolar membrane and enter the lymphatics. Shulz, Warren and Drinker (1938) collected thoracic duct lymph in rabbits after instilling cultures of type III pneumococci into the trachea, and they were able to culture these organisms from the lymph within as short a time as one hour. It would seem, therefore, that some organisms are absorbed directly into the lymphatics without first being ingested by phagocytes; probably more would have been collected from the right lymph duct, had this been cannulated. The chief defence against large numbers of pathogenic organisms, however, is the phagocytic activity of the polymorphonuclear leucocytes which accumulate in the alveoli. By ingesting the bacteria they lessen the absorption into the lymphatics and help to localize the infection

tissue it readily entered the right lymph duct (Drinker and Hardenbergh, 1947). When red cells or the very much smaller protein molecules were introduced through the trachea into the alveoli, however, absorption into the lymphatics of the anaesthetized dog was practically nil These experimental findings led Drinker and Hardenbergh to conclude that the chief barrier to absorption from the alveoli was the alveolar membrane.

Another factor, however, is probably the inadequacy of normal intrapulmonary pressure gradients to propel fluid rapidly from alveoli or interstitial spaces into lymphatics and along them. The intra-alveolar pressure varies little from atmospheric during the normal respiratory cycle with the subject at rest, and pressure changes in the alveolar septa should be similar. In the early stages of pulmonary oedema an excess of interstitial fluid is formed, and only a slight increase in tissue tension is necessary for the spilling over and accumulation of this fluid in the alveoli. Before there is any appreciable accumulation of fluid in the alveoli, the ventilation of these alveoli is normal, and, although histologically the lymphatic vessels are seen to be dilated, there is no appreciable increase in lymph flow from the right lymph duct (Cameron and Courtice, 1946) This suggests that in the absence of appreciable pressure changes within the lung tissue, lymph flow is sluggish, for the excess fluid can quite as easily enter the alveoli as the lymphatics. In the later stages of acute pulmonary oedema when the previously spongy lungs become almost solid, the pressures within these tissues may be increased very considerably by the violent expiratory efforts, and result in high rates of flow of lymph from the right lymph duct. These effects in the various stages of acute pulmonary oedema can readily be observed over a period of several hours in dogs exposed to various doses of phosgene (cf. Cameron and Courtice, 1946)

The lymphatics and pulmonary oedema

The mechanism of production of pulmonary oedema has been the subject of a great deal of experimental work and has been frequently reviewed (cf. Luisada, 1940; Drinker, 1945; Cameron, 1948; Courtice, 1953). Oedema will arise from an increased permeability of the capillary membrane or from an imbalance of the pressures controlling the fluid exchange across this membrane. In all conditions of pulmonary oedema, however, the oedema fluid contains a high concentration of protein. The resolution of pulmonary oedema, however induced, therefore implies the removal from the lungs of fluid containing a considerable amount of protein Large volumes of this fluid may be excreted by coughing, and by ciliary propulsion (Boyd, Perry and Stevens, 1943) In amount this is the most effective way of clearing the lungs of excess fluid.

As far as the lymphatics are concerned, we have seen that in the

Fig. 61 Anaesthesia and the recumbent posture thus have a considerable slowing effect on the removal of protein from the alveoli.

A number of suggestions have been made to explain the sluggishness of lymphatic absorption from the lungs compared with that from the serous cavities. The distance between the nearest lymphatics, in the walls of the alveolar ducts, and the protein-rich fluid in the alveoli may reduce the rate of absorption, especially if intrapulmonary pressures are inadequate to squeeze fluid from alveoli into interstitial tissues or if the alveolar epithelium acts as a barrier to movement of protein into the tissue fluids. That the alveolar epithelium acts as an efficient barrier

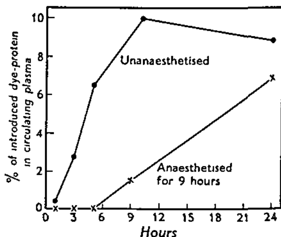


FIG 61 —The concentration of dye in the circulating plasma of rabbits after the introduction into the lungs through the trachea of 1 ml /kg homologous plasma, labelled with the blue dye, T1824

Two groups of animals are represented, one anaesthetized for only a brief period to enable the injection to be made, and the other kept under nembutal anaesthesia for 9 hours and then allowed to recover

(From Courtice and Simmonds, 1949*a*)

would seem unlikely since in pulmonary oedema protein and fluid pass readily into the alveoli from the tissue fluids In the absence of a restriction to protein movement imposed by an alveolar barrier, fluid and protein would probably pass in bulk from alveoli into tissue spaces and lymphatics when pressure differences were adequate. The distance to the nearest lymphatics would matter less in this case than if transfer of protein molecules occurred only by diffusion

Some experiments, however, suggest that the alveolar membrane does act as a barrier Warren and Drinker (1942) raised the pulmonary blood pressure by compressing the pulmonary veins Not only did the lymph collected from a lung lymphatic increase in quantity, but it rapidly became bloody. Red cells escaped from the blood capillaries and readily entered the lymphatics. Also when protein was injected into the lung

oedema, the removal of the protein in the recovery phase is very slow, and in those animals that recover it usually takes about a week for the excess protein to be absorbed from the alveoli. While lymph flow has not been measured during this stage, it is unlikely that it is much above normal, since the breathing is quiet and intrapulmonary pressure changes are minimal. In anaesthetized animals the lymphatic absorption of protein from the alveoli is so slow that Drinker and Hardenbergh (1947) concluded that "unchanged transudates and exudates are removed from the lung alveoli in minute amounts. To clear the lungs of plasma proteins requires breakdown of molecules by enzymatic action until products are formed which are small enough to diffuse readily into the blood". While it is possible that removal by cells or by proteolysis plays a part, the findings by Courtice and Simmonds (1949a) that labelled protein molecules are absorbed for several days, and that in the unanaesthetized animal the absorption of labelled protein is very much greater than in anaesthetized animals, make this hypothesis unnecessary. Fibrinolysins must be important in digesting fibrin, but there is no conclusive evidence that albumin and globulin molecules are also broken down.

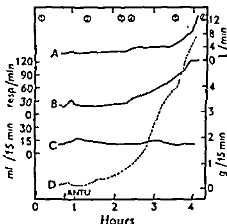


FIG 63—The flow of lymph from the right lymph and thoracic ducts of a dog after the intravenous injection of α-naphthyl thiourea (ANTU), which caused pulmonary oedema

A, respiratory minute volume *B* respiration rate *C*, thoracic duct lymph flow in ml 15 min, *D*, flow of right lymph duct in g 15 min.

(From Drinker and Hardenbergh, 1949)

Central Nervous System

In the formation of cerebrospinal fluid the capillary vessels of the choroid plexuses permit the passage of water, electrolytes and small molecules but hold the blood proteins back These vessels differ from typical capillaries in the fact that fluid leaving them must pass not only through the capillary wall but through a layer of epithelium on the outside of the capillary. Normal cerebrospinal fluid contains but traces of protein. Although lymphatics in their development do not invade the central nervous system, there are certain regions where the cerebrospinal fluid may escape from the meninges and come into close association with

acute stage of oedema formation the lymph flow is increased, especially when areas of the lung become fairly solid with oedema. With exposure to phosgene, after a period of 2 to 4 hours the lymph flow from the right lymph duct began to increase, and at death when the lungs were massively oedematous the lymph flow had risen considerably, Fig. 62. Histologically, the lymphatic vessels were widely dilated and there was no evidence of lymphatic blockage, since in a series of eight experiments

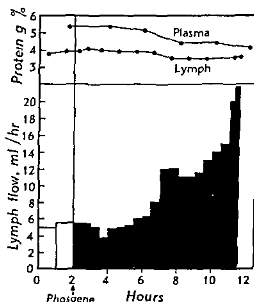


FIG 62 —The flow and protein composition of lymph from the right lymph duct of a dog, after exposure to phosgene The exposure caused massive pulmonary oedema and death in 9½ hours

(Courtice, unpublished)

the lymph flow from the right lymph duct rose on an average from 1 8 to 11·4 ml /hr., a six- to seven-fold increase.

When pulmonary oedema was produced by the application of the arsenical vesicant, lewisite, to the skin or by the intravenous injection of lewisite oxide, similar changes in lymph flow were observed as pulmonary oedema formed (Cameron, Courtice and Short, 1947). Drinker and Hardenbergh (1949) produced pulmonary oedema by the intravenous injection of α-naphthyl thiourea (ANTU) into dogs and found widely-dilated lymphatics and a rise in lymph flow from the right lymph duct, Fig. 63 In these experiments there was not at post mortem the massive oedema observed after phosgene poisoning, but a pleural exudate was usual Some of the increase in lymph flow may have come from the pleural cavities as well as from the lungs themselves

In contrast to the lymph flow in the acute stage of massive pulmonary

oedema, the removal of the protein in the recovery phase is very slow, and in those animals that recover it usually takes about a week for the excess protein to be absorbed from the alveoli. While lymph flow has not been measured during this stage, it is unlikely that it is much above normal, since the breathing is quiet and intrapulmonary pressure changes are minimal. In anaesthetized animals the lymphatic absorption of protein from the alveoli is so slow that Drinker and Hardenbergh (1947) concluded that "unchanged transudates and exudates are removed from the lung alveoli in minute amounts. To clear the lungs of plasma proteins requires breakdown of molecules by enzymatic action until products are formed which are small enough to diffuse readily into the blood". While it is possible that removal by cells or by proteolysis plays a part, the findings by Courtice and Simmonds (1949*a*) that labelled protein molecules are absorbed for several days, and that in the unanaesthetized animal the absorption of labelled protein is very much greater than in anaesthetized animals, make this hypothesis unnecessary. Fibrinolysins must be important in digesting fibrin, but there is no conclusive evidence that albumin and globulin molecules are also broken down.

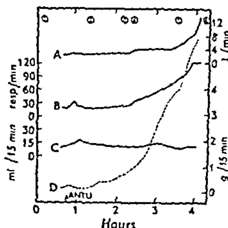


FIG 63.—The flow of lymph from the right lymph and thoracic ducts of a dog after the intravenous injection of α-naphthyl thiourea (ANTU), which caused pulmonary oedema

A respiratory minute volume *B* respiration rate *C*, thoracic duct lymph flow in ml. 15 min *D* flow of right lymph duct in g 15 min.

(*From* Drinker and Hardenbergh 1949)

CENTRAL NERVOUS SYSTEM

In the formation of cerebrospinal fluid the capillary vessels of the choroid plexuses permit the passage of water, electrolytes and small molecules but hold the blood proteins back. These vessels differ from [illegible] nly [illegible] out- [illegible] of protein. Although lymphatics in their development do not invade the central nervous system, there are certain regions where the cerebrospinal fluid may escape from the meninges and come into close association with

nearby lymphatic capillaries. At the head end, it has long been known that cerebrospinal fluid may pass by way of the arachnoid sheaths of the olfactory nerves into the nasal submucosa and ultimately into the cervical lymph ducts (cf. Weed, 1914, 1922; Le Gros Clark, 1929; Faber, 1937, Brierley and Field, 1948). When a dye such as T1824 or Trypan blue is injected into the subarachnoid space one may observe that, after fifteen to thirty minutes, not only does the mucous membrane of the olfactory area become very blue, but that the colour also extends down the deep lymphatics and through the cervical lymph nodes

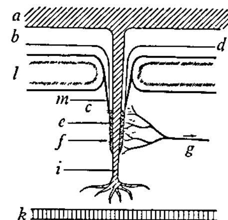


FIG 64—Diagram of olfactory nerve and lymphatic associations in nasal mucosa after injection of India ink into the cerebrospinal fluid in the rabbit

a, olfactory bulb, *b*, sub-arachnoid space, *c*, nasal submucosa, *d*, arachnoid, *e*, ink particles in sub-arachnoid cul-de-sac, *f* ink particles in nasal submucosa, *g*, fine lymph channels draining nasal submucosa, *i*, olfactory nerve, *k*, nasal mucous membrane, *l*, cribriform plate, *m*, dura mater

(From Brierley and Field, 1948)

In these experiments substances with relatively small molecules were used and the injection pressure was not always kept within physiological limits. It has also been conclusively shown, however, that after the introduction into the cerebrospinal fluid, under normal infusion pressures, of India ink particles, 0·4 to 1·5 μ in diameter (Brierley and Field, 1948), of labelled plasma protein (Courtice and Simmonds, 1951), of red blood cells (Simmonds, 1952, 1953*a*) and of thoracic duct chyle containing chylomicrons (Courtice and Morris, 1955), these substances may pass through the cribriform plate into the nasal mucosa where they enter the lymphatic vessels draining into the cervical lymph ducts. The pathway involved is shown diagrammatically in Fig. 64.

The escape of such substances from the spinal and brain-stem subarachnoid is not so apparent, but evidence suggests that injected particles may pass out in the region of the posterior nerve root ganglia and enter the lymphatics in the epidural fat pads. Brierley and Field (1948) and Brierley (1950) have demonstrated the escape of injected India ink particles in these regions especially around the cervical and lumbosacral nerve roots. The subarachnoid space ends near the proximal pole of the posterior root ganglion as a well-defined cul-de-sac in which India ink particles accumulate and from which some of the particles escape. Field and Brierley (1948*a*) by retrograde injection of the lymphatics have traced the lymphatic capillaries from their origin around the posterior nerve root to their

ultimate entry into the thoracic duct, Fig. 65. Using plasma protein labelled with the dye T 1824, Courtice and Simmonds observed sometimes a slight blue staining of the epidural pads of fat and of the lymphatics draining them, but Simmonds (1952) did not obtain evidence of any significant leakage of red cells in these areas.

There are thus two regions where the so-called "leaky-spots" of the arachnoid space occur, along the olfactory nerve fibres into the nose and in association with the sensory ganglia. In the normal animal some of the cerebrospinal fluid escapes this way. Weed (1922) concludes that the absorption of cerebrospinal fluid is "a twofold process, being chiefly a rapid drainage into the great dural sinuses, and in small part a slow indirect escape into the true lymphatic vessels".

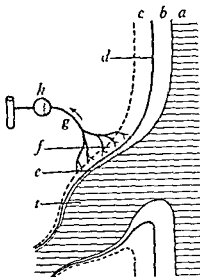


FIG 65.—Diagram of spinal nerve root and associated lymphatics in the rabbit after injection of India ink into the cerebrospinal fluid

a, spinal cord *b* subarachnoid space *c* epidural connective tissue, *d*, dura-arachnoid (rabbit), *e*, ink particles in subarachnoid cul-de-sac, *f*, ink particles in epi-dural connective tissue, *g*, fine lymph channels draining epi-dural tissue, *h*, lymph nodes of body wall, *i*, spinal nerve

(From Brierley and Field, 1948)

These lymphatic associations may be of clinical significance in clearing the cerebrospinal fluid of plasma proteins and cells in meningitis and haemorrhage and in p r o v i d i n g channels of entry for bacteria and viruses into the central nervous system In meningitis the protein concentration in the cerebrospinal fluid may rise considerably. Merritt and Fremont-Smith (1937) in a series of 157 cases of purulent meningitis showed that in two thirds of the patients the protein concentrations were between 100 and 500 mg. per cent, while in a smaller series of 32 patients with meningococcal or pneumococcal meningitis the average protein concentration was 460 mg per cent (Simmonds, 1953*b*) The distribution of protein is not uniform throughout the cerebrospinal fluid, being much higher in the lumbar region than in the cisterna magna and ventricles. Nevertheless, if we assume that the volume of cerebrospinal fluid in man is 90 to 150 ml, there might be in these diseases as much as 500 mg. of protein (equivalent to about 7 ml. plasma) in the cerebrospinal fluid at any one time The amount exuded and removed during the course of the disease would be much greater than this. In subarachnoid haemorrhage the

distribution of red blood cells is less uniform than that of protein, because of the effect of gravity on their sedimentation. The determination of the volume of blood lost in such a haemorrhage is, therefore, difficult, but 10 ml. of free blood would probably be a reasonable estimate. We see, therefore, that although it is difficult to determine the amounts of protein or of red cells exuded into the cerebrospinal fluid in disease, they are probably not inconsiderable.

The experiments described so far define the lymphatic routes for absorption, but do not indicate how much of a naturally-occurring contaminant of the cerebrospinal fluid, such as plasma protein or blood, may be removed in this way. Courtice and Simmonds (1951) introduced into the cisterna magna of the cat dye-labelled plasma protein, to give concentrations in the cerebrospinal fluid of the same order as that observed in patients with meningitis By collecting the lymph from the cervical and thoracic ducts, they showed that in 4 to 5 hours 0·4 per cent of the injected amount of the dye-protein was collected in the thoracic duct lymph, 5 per cent in the cervical duct lymph and 15 per cent in the blood stream The greater part of the injected protein thus entered the blood stream by a route other than by the lymph, an appreciable part passed through the cribriform plate into the nasal lymphatics, but only a negligible amount escaped from the spinal arachnoid. Even this small amount of dye-protein in the thoracic duct could have come from that which entered the blood stream directly and later escaped from the normal capillaries of the alimentary viscera. Similar results were obtained when the labelled protein was injected into the lumbar sac to give high concentrations of dye in this region In rabbits, unanaesthetized except for the introduction of plasma into the subarachnoid space, 0 5 ml./kg. body weight of the animal's own plasma was cleared from the cerebrospinal fluid within 24 hours There is, therefore, a rapid removal from the cerebrospinal fluid of injected protein, and by far the greatest part of this protein is removed by direct absorption into the blood stream

Not only plasma-protein, but red cells also, enter the blood mainly by a pathway not involving the lymphatics (Simmonds, 1952). The position of the head affects the number of red cells appearing in the cervical lymph, more appearing when the animal is kept in the head-down than in the horizontal position. Gravity affects the pooling of red cells, and if this pooling occurs in the anterior fossa, the cervical lymph readily becomes bloody. After the introduction of 0 5 ml /kg. of whole blood into the cisterna magna of rabbits, the cerebrospinal fluid was almost cleared of red cells in 48 hours Similar rates of removal of red cells have been shown in dogs (Sprong, 1934; Meredith, 1941) With repeated injections of whole blood into the cisterna magna of rabbits, three times weekly for three to five weeks, Simmonds (1952) found that

6 to 10 ml. of blood had disappeared from the subarachnoid space leaving virtually no trace. By labelling the red cells with P^{32} before injection, Simmonds (1953*a*) showed in rabbits that on an average 6 *per cent* of injected cells entered the circulation in 5 hours, compared with 20 per cent of labelled protein. Quantitatively the volume of red cells removed [illegible]

the cervical ducts intact or ligated.

The mode of entry of protein and red cells from the cerebrospinal fluid directly into the blood stream is not known for certain. It seems probable, however, that absorption takes place through the arachnoid villi. This is interesting because it is one place where proteins and red cells may return to the blood stream through the vascular endothelium. Sweet and Locksley (1953) also suggest that protein is absorbed largely through the arachnoid villi which serve the function in the cerebrospinal fluid system analogous to the lymphatics of the general circulation. The rapid absorption of protein and red cells into the blood stream can account for the removal of these substances in disease. Whenever there is tissue damage and blood clot, however, removal of fibrin and enmeshed red cells will depend on cellular mechanisms; but when the red cells are free in the cerebrospinal fluid it seems that phagocytosis plays little part in their removal (cf. Simmonds, 1953*b*).

In the eye, too, there are no lymphatics; the capillaries are normally very impermeable to protein and fluid balance is maintained by absorption into blood-vessels. When any protein or particles enter the aqueous humour they may be ingested by the endothelial cells of the iris or hydrolysed to amino-acids by ferments present in the aqueous. Evidence suggests that such materials are not drained away through the canal of Schlemm. For details of fluid exchange in the eye, Davson (1949) should be consulted.

The second major issue is whether or not the lymphatic connexions with the cerebrospinal fluid form channels of entry for bacteria and viruses into the central nervous system. In particular, much attention has for many years been given to the possible entry of neurotropic viruses, such as poliomyelitis virus, from the nose along the olfactory fibres. We have already seen that a solution of potassium ferrocyanide and iron-ammonium citrate will diffuse along this pathway (Le Gros Clark, 1929) and that some bacteria introduced into the nose have been found in the olfactory bulb (Rake, 1937). No conclusive evidence, however, has been produced to demonstrate that neurotropic viruses enter the central nervous system by this route. When one endeavours to force a solution of T1824 from the nasal mucosa up into the cranium, it is practically impossible to

distribution of red blood cells is less uniform than that of protein, because of the effect of gravity on their sedimentation. The determination of the volume of blood lost in such a haemorrhage is, therefore, difficult, but 10 ml. of free blood would probably be a reasonable estimate. We see, therefore, that although it is difficult to determine the amounts of protein or of red cells exuded into the cerebrospinal fluid in disease, they are probably not inconsiderable.

The experiments described so far define the lymphatic routes for absorption, but do not indicate how much of a naturally-occurring contaminant of the cerebrospinal fluid, such as plasma protein or blood, may be removed in this way. Courtice and Simmonds (1951) introduced into the cisterna magna of the cat dye-labelled plasma protein, to give concentrations in the cerebrospinal fluid of the same order as that observed in patients with meningitis. By collecting the lymph from the cervical and thoracic ducts, they showed that in 4 to 5 hours 0·4 per cent of the injected amount of the dye-protein was collected in the thoracic duct lymph, 5 per cent in the cervical duct lymph and 15 per cent in the bloo stream. The greater part of the injected protein thus entered the blooc stream by a route other than by the lymph, an appreciable part passe through the cribriform plate into the nasal lymphatics, but only a negligibl amount escaped from the spinal arachnoid. Even this small amount o dye-protein in the thoracic duct could have come from that which entere the blood stream directly and later escaped from the normal capillaries o the alimentary viscera. Similar results were obtained when the labelle protein was injected into the lumbar sac to give high concentrations o dye in this region. In rabbits, unanaesthetized except for the introductio of plasma into the subarachnoid space, 0·5 ml./kg. body weight of th animal's own plasma was cleared from the cerebrospinal fluid within 2 hours There is, therefore, a rapid removal from the cerebrospinal flu of injected protein, and by far the greatest part of this protein is remove by direct absorption into the blood stream.

Not only plasma-protein, but red cells also, enter the blood mai by a pathway not involving the lymphatics (Simmonds, 1952) Tl position of the head affects the number of red cells appearing in t cervical lymph, more appearing when the animal is kept in the head-dov than in the horizontal position. Gravity affects the pooling of red ce and if this pooling occurs in the anterior fossa, the cervical lymph read becomes bloody. After the introduction of 0·5 ml./kg. of whole blo into the cisterna magna of rabbits, the cerebrospinal fluid was alm cleared of red cells in 48 hours Similar rates of removal of red ce have been shown in dogs (Sprong, 1934; Meredith, 1941). Wi repeated injections of whole blood into the cisterna magna of rabbi three times weekly for three to five weeks, Simmonds (1952) found tl

6 to 10 ml. of blood had disappeared from the subarachnoid space leaving virtually no trace. By labelling the red cells with P^{32} before injection, Simmonds (1953*a*) showed in rabbits that on an average 6 per cent of injected cells entered the circulation in 5 hours, compared with 20 per cent of labelled protein. Quantitatively the volume of red cells removed by the cervical lymphatics was probably very small, for in rabbits, although red cells appear in the cervical lymphatics as in cats, no significant difference could be observed in the absorption of labelled cells in animals with the cervical ducts intact or ligated.

The mode of entry of protein and red cells from the cerebrospinal fluid directly into the blood stream is not known for certain. It seems probable, however, that absorption takes place through the arachnoid villi. This is interesting because it is one place where proteins and red cells may return to the blood stream through the vascular endothelium Sweet and Locksley (1953) also suggest that protein is absorbed largely through the arachnoid villi which serve the function in the cerebrospinal fluid system analogous to the lymphatics of the general circulation The rapid absorption of protein and red cells into the blood stream can account for the removal of these substances in disease. Whenever there is tissue damage and blood clot, however, removal of fibrin and enmeshed red cells will depend on cellular mechanisms; but when the red cells are free in the cerebrospinal fluid it seems that phagocytosis plays little part in their removal (cf. Simmonds, 1953*b*)

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lymphatic vessels in the diaphragm but not to any extent the vessels in other parts of the parietal or visceral peritoneum (cf. von Recklinghausen, 1862; Muscatello, 1895; Durham, 1897; MacCallum, 1903; Buxton and Torrey, 1906; Bolton, 1921; Florey, 1927; Higgins and Graham, 1929; Allen, 1936; Simer, 1944, 1948). Moreover, the absorption of these particles does not take place evenly over the whole diaphragmatic surface but is more extensive on the right side overlying the liver (Higgins and Graham, 1929)

The lymphatics of the diaphragm are arranged as a plexus on the peritoneal surface, the lymph from which drains by intercommunicating vessels into a plexus on the pleural surface. The lymphatic capillaries are not, however, evenly distributed, and the distribution varies in different animals. In man, dog and mouse the lymphatics are practically confined to the muscular portion (MacCallum, 1903, Allen and Vogt, 1937); in the rabbit the tendinous plexus is more important (MacCallum, 1903; Florey, 1927; Allen and Vogt, 1937); in the cat (Higgins, Beaver and Lemon, 1930) and rat (Florey, 1927) a tendinous plexus is absent, in the guinea-pig a tendinous plexus is present, but less important than in the rabbit (Florey, 1927)

After leaving the diaphragm the lymph enters mainly the large collecting ducts which run with the internal mammary blood vessels on either side of the sternum to reach the anterior mediastinal lymphatic nodes in the region of the thymus. From these nodes efferent lymphatic vessels pass, as a rule, to the right side to join the right lymphatic duct In some instances, however, the efferent vessels may enter the veins on the left side, sometimes in association with but often separate from the thoracic duct. This lymphatic route through the anterior mediastinum accounts for about 80 per cent of the lymph from the diaphragm (Higgins and Graham, 1929; Courtice and Steinbeck, 1950*a*) There are subsidiary routes, viz. vessels running up in the mediastinum to the bronchial lymph nodes, and channels emptying directly into the thoracic duct above the diaphragm or running down to lymph nodes in the retroperitoneal fat or the mesenteries and thence to the cisterna chyli In many experimental animals and in man, it seems that the substernal pathway is by far the largest and most important The subsidiary pathways, however, vary in significance in different animals (Brown, 1927–1928, Courtice and Steinbeck, 1950*b*, 1951*a*) It is clear that, in general, the thoracic duct plays a small part in the lymph drainage from the peritoneal cavity Courtice and Steinbeck (1950*a*), by collecting lymph from the right lymph duct and from the thoracic duct of cats, found that on an average only about 20 per cent of labelled plasma protein introduced into the peritoneal cavity passed into the thoracic duct. Again when whole blood was used, most of it was absorbed through the right lymphatic duct and only small amounts

succeed Likewise when dye-labelled plasma protein is injected under pressure into the vertebral column just outside the dura, no dye-protein enters the cerebrospinal fluid (Courtice and Simmonds, 1951).

Field and Brierley (1948*b*) have discussed their experiments as an anatomical basis for invasion of the nervous system along direct lymphatic communications. Since poliomyelitis virus is now thought to enter the body through the alimentary tract, the olfactory pathway is regarded as being of less significance for the mode of entry of this virus into the brain than it was earlier. The retrograde filling of the lymphatics draining the posterior root regions, after rendering the valves incompetent by ligating the thoracic duct, has suggested that perhaps viruses could enter the spinal cord along these lymphatics in certain circumstances. No direct evidence, however, has as yet been produced to prove this.

The Serous Cavities

The serous cavities—peritoneal, pleural and pericardial—are all derived in the embryo from one body cavity and their functions and reactions are similar. Each cavity is lined by a membrane which is reflected everywhere over the viscera. This mesothelial lining consists of a layer of flat, pavement cells supported by a basement membrane of connective tissue. Any fluid or other materials in a serous cavity must, therefore, pass through the mesothelial lining and the supporting connective tissue before reaching the lymphatic or blood capillaries. In studying the physiological significance of the lymphatic plexuses which drain the serous cavities, we must consider not only their function in the normal animal but also their capacity to clear these cavities of abnormal accumulations in times of emergency Large molecules such as the plasma proteins which are present in all effusions or exudates readily enter the lymphatic capillaries once they pass through the mesothelium, while particles such as red cells or bacteria may be ingested by phagocytes or enter the lymphatic vessels in the free state. For detailed reviews of the physiology of the serous membranes reference should be made to Cunningham (1926) and Courtice and Simmonds (1954)

The Peritoneal Cavity

Lymphatic pathways of absorption

Although the peritoneum is extensive, lymphatic absorption of substances injected into the peritoneal cavity takes place almost wholly through one small area, the surface of the diaphragm When a suspension of inert particles, such as India ink, hydrokollag 300 or colloidal silver, is introduced into the peritoneal cavity, these particles may be seen to enter the

such as milk or casein (Kjöllerfeldt, 1917) or with such substances as phenolsulphonphthalein, indigo-carmine and potassium ferrocyanide which enter the blood capillaries by diffusion (Starling and Tubby, 1894, Meltzer, 1897; Mendel, 1899; Dandy and Rowntree, 1914, Shipley and Cunningham, 1916*b*) in anaesthetized animals and on the assumption that the diaphragmatic lymphatics drained almost entirely into the thoracic duct, have led to the erroneous conclusion that the lymphatic removal of fluid from the peritoneal cavity is slow and is not responsible for absorption of significant amounts (Cunningham, 1926, Watkins and Fulton, 1938)

The naturally occurring fluids in the peritoneal cavity resemble plasma in composition, but with varying protein concentrations In normal animals Maurer, Warren and Drinker (1940) found average protein levels in the peritoneal fluid collected to be 2 56 per cent in the dog, 1 84 per cent in the rabbit and 2·54 per cent in the cat. In pathological states in human beings average protein levels in the ascitic fluid were 1·2 per cent in cases of cirrhosis of the liver, 3·1 per cent in heart failure and 3·6 per cent in carcinoma (James, 1949). Inflammatory exudates contain protein in higher concentrations approximating those in plasma The natural function of the diaphragmatic lymphatics is, therefore, to remove a fluid containing variable concentrations of plasma protein, and occasionally, when haemorrhage occurs into the peritoneal cavity, to remove red cells as well.

In unanaesthetized experimental animals the capacity of the diaphragmatic lymphatics to remove such fluids is considerable, Fig 66 Courtice and Steinbeck (1950*b*, 1951*a*) found that 20 ml kg of homologous plasma could be removed from the peritoneal cavity of the rat in 3 to 5 hours, the rabbit in 8 hours and the guinea-pig in 16 to 24 hours and that whole plasma was removed as quickly as or even more quickly than isotonic sodium chloride or Ringer-Locke Solution These results are in general agreement with similar experiments done on larger animals (Orlow, 1895; Bolton, 1921). Hypotonic salt solutions are of course initially absorbed much more rapidly by diffusion of water into the blood vessels, but once equilibrium is reached and the fluid becomes isotonic with normal tissue fluid, the rate decreases (Clark, 1920–1921) When any fluid is introduced into the peritoneal cavity, equilibrium between the smaller, readily diffusible molecules and electrolytes of the blood and injected fluid is rapidly attained, but the protein level reaches an equilibrium much more slowly. If a fluid of high protein content is used the concentration of protein in the peritoneal fluid falls, whereas when fluid with a little or no protein is introduced, the protein level rises; but before a steady level is reached all the peritoneal fluid is generally absorbed (Courtice and Steinbeck, 1950*b*; 1951*a*) While the process of diffusion is proceeding the diaphragmatic lymphatics will be absorbing as a whole whatever fluid

through the thoracic duct (Courtice, Harding and Steinbeck, 1953). Abdou, Reinhardt and Tarver (1952), however, were able to collect from the thoracic duct the greater part of the labelled protein which they had introduced into the peritoneal cavity of the rat.

Although there is now no doubt that lymphatic absorption of materials free in the peritoneal cavity takes place almost entirely through the diaphragm, the part played by the omentum has always been a subject for debate. Buxton and Torrey (1906) observed in the guinea-pig after the intraperitoneal injection of lamp-black that the lymphatics of the omentum took up particles, although phagocytosis by the macrophages on the surface of the omentum was a more prominent feature. The particles became entangled in a deposit of fibrin before being engulfed. The action of the macrophages which are present throughout the omentum both as scattered cells and as round or oval aggregates, called "milky spots", forms in itself an efficient defence mechanism against bacteria. While some have doubted the existence of omental lymphatics (Shipley and Cunningham, 1916*a*, Seifert, 1923), others have demonstrated clearly a fairly rich plexus of lymphatics, generally associated with the blood vessels, in the omentum of cats, rabbits, dogs, rats and man, the lymph from which enters the cisterna chyli or thoracic duct (Casparis, 1918–19, Simer, 1934*a* and *b*, 1935) Although there appears to be no doubt that the omentum contains lymphatics, the amount of absorption by these vessels is in no way comparable with that of the diaphragm; nor does the removal of the omentum affect the absorptive capacity of the diaphragm (Higgins, Beaver and Lemon, 1930, Higgins and Bain, 1930, Simer, 1944).

Rate of lymphatic absorption

Experiments with particles have indicated that suspensions introduced into the peritoneal cavity may enter the diaphragmatic lymphatics exceedingly rapidly. For example, Florey (1927) found that all the diaphragmatic lymphatics were evenly injected and the mediastinal lymph glands were jet black five minutes after the intraperitoneal injection of hydrokollag 300 into a rabbit. In the anaesthetized dog Higgins, Beaver and Lemon (1930), by means of a window in the lateral wall of the chest, observed that particles introduced into the peritoneal cavity appeared in the pleural lymphatics of the diaphragm within two to three minutes. These experiments, however, do not tell us the quantity of these materials which the lymphatics are capable of removing.

The natural materials which the lymphatics are generally called upon to absorb, moreover, are not foreign inert particles, but ascitic fluid, inflammatory exudates or red cells The rate at which such fluids as these can be absorbed gives an indication of the normal function or reserve of function of the diaphragmatic vessels Experiments with foreign proteins

such as milk or casein (Kjöllerfeldt, 1917) or with such substances as phenolsulphonphthalein, indigo-carmine and potassium ferrocyanide which enter the blood capillaries by diffusion (Starling and Tubby, 1894; Meltzer, 1897; Mendel, 1899; Dandy and Rowntree, 1914; Shipley and Cunningham, 1916*b*) in anaesthetized animals and on the assumption that the diaphragmatic lymphatics drained almost entirely into the thoracic duct, have led to the erroneous conclusion that the lymphatic removal of fluid from the peritoneal cavity is slow and is not responsible for absorption of significant amounts (Cunningham, 1926; Watkins and Fulton, 1938)

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is presented to them, protein molecules as readily as the smaller electrolytes and water.

The introduction of a foreign plasma into the peritoneal cavity to determine the power of the lymphatics to remove fluid may lead to an erroneous conclusion. A factor, which may be in the lipoprotein fraction of the plasma, causes a temporary outpouring of a protein-rich fluid into the peritoneal cavity by damaging the blood capillary wall. If the foreign plasma is allowed to age for some weeks in the refrigerator, however, this factor is not so effective and the foreign plasma is absorbed as rapidly as homologous plasma (Courtice and Steinbeck, 1951*a*; Lake, Simmonds and Steinbeck, 1953*a* and *b*).

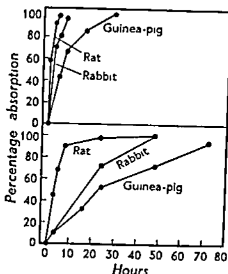


FIG 66—The rate of absorption from the peritoneal cavity of 20 ml/kg homologous plasma (upper diagram) and of red cells from 20 ml/kg homologous blood

Absorption is expressed as a percentage of the volume introduced into the peritoneal cavity

(From Courtice, Harding and Steinbeck, 1953)

Not only diluted or whole plasma but also whole blood can be removed fairly rapidly from the peritoneal cavity. Florey and Witts (1928) by collecting lymph from the thoracic duct before and after the intraperitoneal injection of whole blood in cats were not impressed by the power of the lymphatics to remove red cells from the peritoneal cavity. When the right lymph duct in these animals is cannulated, however, it is found that most of the red cells are absorbed along this pathway, Table 26 It is remarkable to watch the clear lymph from this duct rapidly resemble whole blood with a red cell count rising to 4 to 6 million per mm.^{3}, while the thoracic duct lymph becomes only faintly pink The red cells in the lymph appear perfectly normal and do not undergo haemolysis. It has also been shown in other ways that the red cells enter the blood stream intact. Hahn *et al* (1944) and McKee and Stewart (1950) used red cells with radio-active iron incorporated in the haemoglobin, while Hedenstedt (1947) used blood containing elliptocytes.

In the unanaesthetized animal, 20 ml./kg. of whole blood can be removed from the peritoneal cavity of the rat in 24 hours, rabbit in 48 hours and guinea-pig in 72 hours. The plasma is absorbed more rapidly than the red cells leading to a progressive concentration of the injected blood

TABLE 26

The red cell count in the lymph from the right lymph duct and thoracic duct of a cat before and after the intraperitoneal injection of 10 ml./kg. homologous blood containing 8,020,000 red cells/c mm

Time	Air breathed	Right lymph duct: Lymph flow ml./hr.	Right lymph duct: Red cells in lymph/c mm.	Thoracic duct: Lymph flow ml./hr.	Thoracic duct: Red cells in lymph/c mm.
Before	Room air	0·7	80	7·5	450
After					
0–¼ hr.	Room air	0·8	90	5·2	1,300
¼–½ hr.	,,	0·8	503,000	—	—
½–¾ hr.	,,	0·8	2,650,000	7·2	1,625
¾–1 hr.	,,	1·2	3,680,000	7·2	1,950
1–1½ hr.	,,	1·0	4,170,000	8·0	2,180
1½–2 hr.	,,	1·0	4,530,000	7·0	5,700
2–2¼ hr.	5 p.c. CO_2	2·8	5,550,000	14·4	68,000
2¼–2½ hr.	,,	4·4	5,560,000	14·4	90,000
2½–3 hr.	Room air	2·0	5,550,000	3·4	108,000
3–3½ hr.	,,	2·2	4,840,000	4·8	93,500

From Courtice, Harding and Steinbeck (1953)

and ultimately to a sticky layer of red cells covering the entire visceral and parietal peritoneum. Within the times stated above, however, this sticky mass of cells is absorbed by the lymphatics, leaving no trace of the injected blood in the cavity. In larger animals Hahn *et al.* found that 20 to 100 per cent of the injected red cells (20 to 25 ml. in dogs) were absorbed in 24 hours.

Whenever any fluid is introduced into the peritoneal cavity, there is a cellular response in which several types of cells, but mainly neutrophil leucocytes and large mononuclear macrophages accumulate in the free fluid (cf. Buxton and Torrey, 1906; Webb, 1931–1932). These phagocytes play a part in the removal of particulate matter. Buxton and Torrey described in guinea-pigs how, after the intraperitoneal injection of chicken red corpuscles, the macrophages became stuffed full of these cells. Quantitatively, however, although some of the red cells are ingested by macrophages while in the peritoneal cavity, and further macrophages engorged with red cells are seen in the anterior mediastinal lymph nodes, the number ingested is negligible compared with those passing intact through the lymphatics into the blood stream. No significant difference is observed in the rise in the haemoglobin concentration of the circulating blood in groups of rats given blood intravenously and intraperitoneally (Courtice, Harding and Steinbeck, 1953). It is remarkable how readily the red cells pass through the lymph nodes.

The ease with which free red cells are absorbed from the peritoneal cavity by the lymphatics and ultimately reach the blood stream intact has suggested this route for blood transfusions in certain cases, especially in

children (Siperstein and Sansby, *1923* ; *Siperstein, 1923* ; *Cole and Montgomery*, 1929 ; Clausen, 1940)

Although red cells nearly all pass into the lymphatics, bacteria are more readily ingested locally by phagocytes. Durham (1897) and Buxton and Torrey (1906) have shown that bacteria may be destroyed rapidly by phagocytosis, especially in the omentum, in which case few organisms are absorbed into the lymphatics, or more slowly, when larger numbers of bacilli are absorbed through the lymphatics into the blood stream to be deposited in various organs of the body. In man, Durham showed that in 30 cases of peritonitis the mediastinal lymph nodes contained bacteria. The lymphatics of the diaphragm in these inflammatory conditions are apparently not blocked by thrombi as is the case often in localized inflammatory lesions Bangham, Magee and Osborn (1953) studied the lymphatic *absorption of radio-active glass particles containing caesium*^{134} from the peritoneal cavity of the rat and found that peritonitis produced by turpentine caused no mechanical obstruction to the lymphatic drainage of these particles through the diaphragmatic vessels.

Mechanism of lymphatic absorption

The rapidity with which large particles up to the size of red blood cells gain access to the diaphragmatic lymphatics has for many years aroused interest in the actual mechanism of absorption. Three stages in absorption must be considered ; movement to the absorbing surface, passage across the peritoneal mesothelium and the lymphatic endothelium, and propulsion along the lymphatic channels. In all three stages the movements and changes in pressure associated with respiration, particularly with the excursions of the diaphragm, are of paramount importance. Experimentally, these effects have been shown in many ways

One of the reasons why it was once believed that the lymphatic removal of fluids from the peritoneal cavity was slow, was that experiments were done on anaesthetized animals Florey (1927) showed that in the anaesthetized rabbit, the absorption of *hydrokollag particles through the diaphragmatic* lymphatics was very much slower than in an unanaesthetized animal. Morris (1953) by measuring the rate of absorption of plasma proteins labelled with the dye T1824 and of red cells labelled with P^{32}, has shown that in the rat nembutal anaesthesia reduces the rate of absorption to only 20 to 40 per cent of normal, Fig 67. This effect of anaesthesia is probably due partly to decreased activity of the diaphragm (Davis and Morris, 1953) and partly to decreased intra-abdominal pressure caused by flaccidity of the abdominal muscles Paralysis of the diaphragm produced by acute phrenic *neurectomy also gives rise to a considerable* decrease in the rate of absorption of particles in the dog (Higgins, Beaver and Lemon, 1930) and of labelled protein and of red cells in the rat

(Morris, 1953) In both these groups of experiments the rate of absorption increased to normal or above, two or more weeks after neurectomy when the diaphragmatic muscle became atrophied. Higgins *et al* considered that progressive atrophy of the muscle tissue facilitated the passage of particles through the diaphragm and thus hastened absorption Morris, however, associated the return to normal with the changes in respiratory function. After phrenic neurectomy the accessory muscles of respiration,

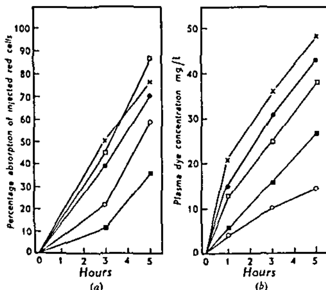


FIG 67—The effect of alterations in respiratory movement on the absorption of red cells labelled with P^{32} and of plasma protein labelled with T1824 from the peritoneal cavity of the rat

(Redrawn from Morris, 1953)

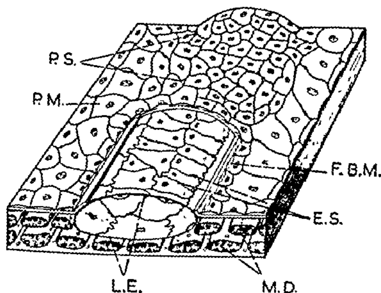
intercostal and abdominal muscles, increase enormously in activity and in about 2 weeks these muscles are able to restore the pulmonary ventilation and the intrapleural pressure to normal limits (Davis and Morris, 1953) It therefore seems likely that the excessive activity of these accessory muscles causes sufficient passive movement of the diaphragm, especially where it is attached to the ribs, to increase the lymphatic absorption Increased respiratory activity in the normal animal induced by the breathing of 5 per cent CO_2 also causes a great increase in lymphatic absorption by the diaphragm (Courtice and Steinbeck, 1950*a*, Morris, 1953).

Diaphragmatic movement, then, plays a major rôle in the transfer of materials within the peritoneal cavity to the peritoneum covering this muscle; but there are probably other factors involved, too. Since lymphatic absorption from the peritoneal cavity occurs mainly through the diaphragm, posture would seem to play an important part. Fowler (1900) made use of this fact in his treatment of peritonitis in human patients by posture. He maintained that in the pelvis the lymphatics were fewer than in the diaphragm and soon became blocked with thrombi, so that, if the fluid could be kept in the pelvis, absorption would be minimized. The absorption of labelled protein (Courtice and Steinbeck, 1951*b*) and of radio-active glass particles (Bangham, Magee and Osborn, 1953) are initially slowed when the animals are kept in the pelvis-down position. It is remarkable, however, how quickly in this position some of the materials injected into the pelvis will reach the diaphragm and be absorbed. Some forces cause a drift of material, even of a layer of sticky red cells adhering to the whole of the visceral and parietal peritoneum, to the under surface of the diaphragm against the force of gravity. The movements of the intestines as well as those of the diaphragm probably have a mixing effect on any contents of the peritoneal cavity. Higgins, Beaver and Lemon (1930) showed that, in dogs, opening the abdomen and handling the intestines delayed the arrival of graphite particles at the diaphragmatic surface, and paralytic ileus in rabbits delayed the movement of radio-opaque material from the site of injection (Efskind, 1940).

The manner in which particles, having reached the diaphragm, pass through the mesothelium has led to much controversy since von Recklinghausen (1863) gave evidence for the existence of stomata between the cells, through which the particles passed. He placed ink, milk and other substances on the peritoneal surface of the excised diaphragm, and under the dissecting microscope observed vortices in the suspensions in the region of the so-called stomata, and a flowing of the absorbed material along subserous lymphatics. This concept of the anatomical arrangement of the diaphragmatic peritoneum could not be confirmed by Kolossow (1893), Muscatello (1895) or MacCallum (1903). MacCallum describes the lymphatic vessels on the peritoneal surface of the diaphragm as running for the most part parallel with one another in the connective tissue between the muscle bundles. These lymphatic canals are abundantly connected by anastomosing channels which run obliquely or transversely across the muscle bundles, often arching toward the peritoneum to form sac-like channels or lacunae. The roof of such a lacuna consists of the endothelial lining of the lymphatic and the mesothelium of the peritoneum with only a lattice of fibrils as a basement membrane, Fig. 68. Elsewhere there is a complete basement membrane to the mesothelial cells which impedes the passage of particles into the lymphatic vessels.



(*a*)



(*b*)

FIG 68 —Lymphatic capillary on peritoneal surface of diaphragm.

(*a*) Microphotograph, showing the large lymphatic lacuna immediately beneath the peritoneum M muscle of diaphragm, L, lymph vessel, S, peritoneal lining

(From Fig 4, Simer, P H, 1944 *Anat Rec*, 88, 1823)

(*b*) Diagrammatic representation of a lymphatic lacuna lying immediately beneath the peritoneum

F B M = Fenestrated basement membrane
E S = Endothelial stigmata
P M = Peritoneal mesothelium
P S = Peritoneal stomata
L E = Lymphatic endothelium
M D = Muscle of diaphragm

Note that the pointer for the fenestrated basement membrane does not quite reach it

(From Plate 1, Allen, L A 1936 *Anat Rec* 67, 101)

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stretching, as in relaxation, increased the volume of the lymphatics, brought about by a pulling action on the connective tissue fibres attached to the walls of the vessels Because of the resultant decreased intralymphatic pressure, fluid and particles entered from the peritoneal cavity, but there was no reflux from the larger lymphatic channels because of valves. When the stretch on these fibres is diminished, as in diaphragmatic contraction, the lymphatic capillaries decrease in size and the lymph they contain is expelled forward into the larger valved vessels

Besides the effect of contraction and relaxation of the diaphragm on the volume of the lymphatics, intra-abdominal pressure is also important, for it is the gradient between the pressure immediately under the diaphragm and the pressure within the lacunae which provides the force for absorption The intra-abdominal pressure has been determined in animals by the insertion of balloons under the diaphragm and has been shown to vary greatly in different conditions such as anaesthesia, posture, coughing, vomiting and straining (Florey, 1927, Drye, 1948, Lam, 1939) The effect of intra-abdominal pressure on the entrance of materials into the lymphatic lacunae is, therefore, probably very variable in an unanaesthetized animal, and it cannot be determined with accuracy.

The evidence for the passage of materials between the mesothelial cells concerns particles only There is no direct evidence that protein molecules and the smaller molecules and electrolytes present in peritoneal fluid pass between these cells. In the previous chapter, however, evidence suggests that these substances pass through the blood capillary membrane between the cells, so by analogy it would seem reasonable to believe that they would also pass between the mesothelial cells of the peritoneum and the endothelial cells of the lymphatics, and not through their cytoplasm. The fact that red cells and plasma protein molecules are both rapidly absorbed at rates not proportional to their size suggests that these substances are passing through gaps of considerable size relative to the protein molecule Morris (1956*b*) has shown, too, that fat particles up to 1 μ in diameter are absorbed from the peritoneal cavity as rapidly as plasma protein Yet McKee *et al* (1952) found that albumin left the peritoneal cavity more rapidly than globulin in ascitic dogs This would suggest that the protein is passing through submicroscopic pores, in which case it is not easy to explain why isotonic Ringer-Locke solution is not absorbed more quickly than plasma

Lymph propulsion

Once the fluid enters the lymphatics it is propelled along the lymphatic trunks mainly by forces set up by respiratory movement. These forces will be considered in more detail in the next chapter.

During the passage of the lymph along the large trunks in the thoracic

One reason for the easy passage of particles through the diaphragmatic peritoneal barrier is, no doubt, the absence of a complete basement membrane. To explain the passage through the roof of a lacuna, MacCallum postulated that "the connexions of the endothelial cells are so lax that the violent pumping action of the respiratory movement is enough to force material between them when they come to form the only obstruction to its entrance. In reality, this is very near practically to von Recklinghausen's idea of stomata, but it differs in holding the peritoneal epithelium as a complete layer of cells distinct from the lymphatic endothelium and not passing over into them through any communicating channel."

Since the work of MacCallum, the manner in which large particles so easily gain access to the diaphragmatic lymphatics has intrigued many anatomists and physiologists. Cunningham (1926) in reviewing this subject wrote "The earlier work all tended to establish the concept of the presence of actual preformed physical openings between the peritoneal cavity and the diaphragmatic lymphatics. This idea was gradually eliminated, and in its place the concept of potential physical openings between cells was offered. In turn this hypothesis is being replaced by one which assumes that most, if not all, the particulate material that is absorbed from the peritoneal cavity passes directly through the living cytoplasm of the mesothelial cells." This latter concept seems most unlikely. Florey (1927) and, later, Simer (1944, 1948) confirmed MacCallum's observation that the cell boundaries of the diaphragmatic peritoneum are outlined by carbon particles during absorption with little evidence of passage through the cytoplasm Allen (1936) went a step further back towards von Recklinghausen's concept of preformed stomata

In the investigation of the presence or not of preformed stomata, the maximum size of particles that can be absorbed has been determined. All [illegible] gain access. [illegible] ameter were [illegible] lei, 7–9 μ in diameter did not enter. These cells were fixed and stained before injection Courtice, Harding and Steinbeck (1953) observed that in the rat, fresh rat, rabbit or human red cells ranging in diameter from 6–8 μ readily entered the lymphatics. Maybe fresh red cells could by their elasticity pass through spaces which fixed cells could not.

It is now generally accepted that particles up to about 7 μ in diameter pass through the mesothelium between the cells, but the question of preformed stomata or of potential spaces caused by the contraction and relaxation of the diaphragm is still debatable. The forces required for fluid and particles to enter the lymphatic lacunae have been discussed by Allen and Vogt (1937). In the excised diaphragm they showed that

stretching, as in relaxation, increased the volume of the lymphatics, brought about by a pulling action on the connective tissue fibres attached to the walls of the vessels. Because of the resultant decreased intralymphatic pressure, fluid and particles entered from the peritoneal cavity, but there was no reflux from the larger lymphatic channels because of valves. When the stretch on these fibres is diminished, as in diaphragmatic contraction, the lymphatic capillaries decrease in size and the lymph they contain is expelled forward into the larger valved vessels.

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cavity, some fluid escapes into the mediastinal tissues and the pleural cavity (Courtice and Steinbeck, 1950*b*, 1951*a*). When whole blood is being absorbed, red cells also sometimes escape. The points of exit of the fluid and cells appear to be in the smaller tributaries which drain into the large channels. When the lymphatics are functioning maximally and are widely dilated, the negative pressure in the thorax together with the resistance of the lymph nodes to flow cause the lymph to escape along the tributaries in the parietal pleura and into the mediastinum. When foreign plasma is used, the escape into the pleural cavity may be very considerable. Ultimately the fluid that escapes into the pleural cavity and mediastinum is absorbed by the local lymphatics When the parasternal lymphatic trunks are obstructed by a ligature passed around the sternum to include these vessels, fluid introduced into the peritoneal cavity and absorbed into the diaphragmatic lymphatics escapes from the lymph trunks into the pleural cavity in the same way (Courtice and Steinbeck, 1951*b*). From a practical viewpoint it seems that fluid in the pleural cavity may sometimes originate in this way from the peritoneal cavity.

So far we have considered the capacity of the lymphatics to deal with the introduction of a fairly large quantity of isotonic fluid containing plasma protein, of whole blood or of a suspension of particles. These experiments give an indication of the functional significance of the peritoneal lymphatics in an emergency, such as the resolution of an inflammatory or traumatic exudate Another common pathological condition is ascites, in which it is now realized that there may be a very considerable turnover of fluid and of protein. In such a condition the lymphatics must play an important rôle. McKee, Whipple and their co-workers found that the exchange between plasma and ascitic fluid of C^{14}-labelled plasma proteins was very high in dogs actively forming ascitic fluid after constriction of the inferior vena cava above the diaphragm, e g. about 6 per cent of circulating albumin passed each hour from the blood stream to ascitic fluid and similar amounts left the peritoneal cavity, presumably by way of the lymphatics. Volwiler, Grindlay and Bollman (1950) found a somewhat slower turnover in experimental ascites produced by plasmaphaeresis and constriction of the portal vein and inferior vena cava.

In ascites, the lymphatics are important, not only in the part they play in returning protein and fluid to the blood-stream, but also in the production of the ascitic fluid Bolton and Barnard (1931) observed greatly distended lymphatics under the liver capsule in experimental ascites in cats, but they considered that fluid leaked into the peritoneal cavity from the pericapillary spaces rather than through the lymphatic walls. Volwiler *et al.* showed that liver lymph flow was greatly increased in this condition and they postulated that lymph leaked from the hilar and subcapsular lymphatics as well as tissue fluid through the liver capsule.

Hill *et al.* (1953) suggested that ascites in serous hepatosis, a liver disease in Jamaican children, may also be produced in this way. This would mean that one of the sources of ascitic fluid is the lymphatic vessels of the liver, while the fluid is at the same time being rapidly absorbed by another set of lymphatics, those of the diaphragm.

The Pleural Cavity

Lymphatic pathways of absorption

As in the peritoneal cavity, the absorption of colloidal molecules and of particles from the pleural sacs takes place in certain areas in preference to others. In considering the physiological function of the pleural lymphatics, the pleura may be divided into the visceral pleura covering the lungs, the parietal pleura adherent to the intercostal muscles and ribs, the diaphragmatic pleura covering the superior surface of the diaphragm and the mediastinal pleura. The cephalic portion of the mediastinal pleura is closely adherent to the cellular tissue surrounding the trachea, oesophagus, and great vessels, while caudally between the heart and diaphragm are two folds of pleura, one on each side of the midline, reflected from the diaphragm to the pericardium and pulmonary roots

The lymphatics of the pleura covering the lungs are arranged as a single plexus, the smaller vessels uniting to form a variable number of trunks which drain into the lymph nodes at the hilum after passing around the surface of the lung Within the pleura the lymphatics are provided with valves which open in all directions, thus allowing free circulation of lymph within the limits of the pleura These superficial vessels are in communication with the deeper pulmonary lymphatics in the interlobular septa which arise from the pleura. The movement of lymph from the pleural vessels through these communications with the deep vessels depends upon the presence or absence of valves in the communicating vessels Miller (1937) maintains that the valves in these vessels point towards the pleura, which precludes the flow in the pleural lymphatics from entering the lungs. Others, however, maintain that lymph can flow [illegible] round-

[illegible] 1932;

The lymphatics of the parietal pleura exist as a plexus over the muscles, but not covering the ribs, with collecting trunks running at the upper and lower borders of each intercostal space, Fig. 69. These larger channels drain the lymph in part ventrally to the parasternal lymph trunks accompanying the internal mammary blood vessels, and in part dorsally to vessels which run cephalically to upper mediastinal lymph nodes The vessels of the pleural surface of the diaphragm collect the lymph from the peritoneal surface and drain into the large parasternal lymphatics, which

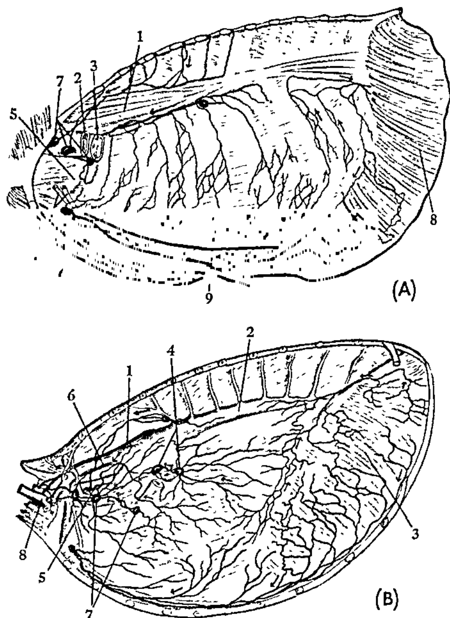


FIG 69—Diagrammatic representation of the lymphatics of (*A*) the parietal and (*B*) the mediastinal pleura in the dog

(*A*)—1, M longus colli, 2, Oesophagus, 3, Trachea, 4, Internal mammary artery and vein, 5, First rib; 6, Sternal lymph gland, 7, Cranial mediastinal lymph glands, 8, Diaphragm, 9, M transversus thoracis (cut)

(*B*)—1, Thoracic duct, 2, Aorta, 3, Diaphragm; 4 Lymph glands of the bifurcation, 5, Brachiocephalic artery, 6, Left subclavian artery, 7, Cranial mediastinal lymph glands, 8, Junction of subclavian, axillary and jugular veins

(From Baum, 1918)

ultimately enter the upper anterior mediastinal lymph nodes near the thymus. The upper mediastinal pleura drains cephalically into nearby lymph nodes. The pleural folds between the heart and diaphragm contain lymphatics which apparently drain cephalically to upper mediastinal lymph nodes, Fig. 69 These folds are important in the absorption of particles because of their incessant movement and stretching and because they contain Kampmeier foci (Kampmeier, 1928*b*; Cooray, 1949) These foci are clusters of cells traversed by blood vessels derived from the fairly large trunks of the adjacent fatty lobules. The cells are phagocytic in function.

Lymphatic absorption from the pleural cavity occurs mainly through two regions of the pleura, the lower mediastinal folds and the parietal intercostal pleura. In contrast to the peritoneal aspect of the diaphragm, the pleural aspect has little absorptive capacity, nor does the visceral pleura covering the lungs seem of much importance in removing free particles or fluid from the pleural sacs (Lemon and Higgins, 1932*b*; Courtice and Simmonds, 1949*b*, Cooray, 1949, Courtice and Morris, 1953).

The relative importance of the mediastinal and of the intercostal pleura in absorption is difficult to assess. When dye-labelled plasma proteins or suspensions of particles are injected into the pleural cavities, the mediastinal pleura is the site of deepest staining or of adhesion of particles In Cooray's experiments practically all the particles followed this course irrespective of the posture of the animal; the costal, diaphragmatic and visceral pleura played only a minor part. With dye-labelled plasma proteins (T1824 in plasma), however, the intercostal lymphatics of the parietal pleura were also deeply outlined in blue, although the lower mediastinal region appeared to be the region of maximum accumulation (Courtice and Simmonds, 1949*b*; Courtice and Morris, 1953)

The ultimate pathways for the lymph draining the pleural cavity are the right lymph duct and the thoracic duct Of these, in the cat, more labelled plasma protein passes into the right lymph duct than into the thoracic duct following intrapleural injection (Courtice and Simmonds, 1949*b*). Wrong conclusions may, therefore, be drawn if lymph from the thoracic duct only is considered.

Mechanism of absorption

Karsner and Swanbeck (1921) considered that, in anaesthetized cats, particles in the pleural cavity moved under the influence of gravity Cooray (1949), however, found in rabbits, rats and guinea-pigs that injected fluid, colloid and particles followed an invariable course, caudally towards the diaphragm and then cephalically along the mediastinal pleural folds to the lung roots. Posture had no effect on the direction of spread, but paralysis of the diaphragm caused a considerable delay. He concluded

that the movement was due to an aspirating effect of the diaphragm on inspiration greater than propulsion in the reverse direction during expiration

The mechanisms involved in the entry of material into the lymphatics are probably similar to those suggested for the peritoneal cavity. Dybkowsky (1866) considered that in rabbits and dogs particles were mainly absorbed into the intercostal lymphatics through permanent stomata The terminal lymphatics were filled during inspiration when the inward drag of the elastic lungs on the pleura and the outward drag of the intercostal muscles pulled the walls of the lymphatics apart and material was sucked in During expiration the tension was relaxed and the superficial lymphatics collapsed, expressing their contents into the deeper efferent vessels It is now believed that communications between lymphatics and pleural cavity, if they exist, are not permanent but are due to temporary dehiscence of adjoining mesothelial cells when the pleura is stretched Allen and Vogt (1937) showed that the intercostal lymphatics could be filled by placing carbon suspension over the pleural aspect of excised parts of the chest wall and stretching the tissues. Cooray observed particles in the cytoplasm of the mesothelial cells and concluded that the particles passed both between the mesothelial cells and through their cytoplasm. In killed animals with artificial respiration the particles then entered the lymphatics, but in living animals all the particles were ingested by polymorphs or the macrophages of Kampmeier's foci, and the lymph nodes showed no particles. Karsner and Swanbeck considered that most of the particles were ingested by phagocytes. Although they invariably found pigment in the lymph nodes of the mediastinum, this pigment was always in mononuclear cells in the lymph sinuses.

The evidence suggests, therefore, that particles such as India ink and killed or living bacteria introduced into the pleural cavities may be ingested by polymorphs in the pleural sacs or may pass mainly through the inferior mediastinal folds of pleura where they are taken up by the macrophages of the specialized Kampmeier's foci. As far as the lymphatics are concerned, some of the particles entering the mediastinal folds may enter the lymphatic vessels and appear in macrophages in the lymph nodes, while others may enter the intercostal lymphatics. Cooray has ascribed to the Kampmeier foci a defensive function to prevent the spread of bacteria through the mediastinum.

Although bacteria and foreign inert particles such as India ink are largely ingested, especially in Kampmeier's foci, plasma protein molecules and red cells fairly rapidly pass into the lymphatic vessels. Some of the red cells will be engulfed by the macrophages of Kampmeier's foci and of the lymphatic nodes, as in the absorption from the peritoneal cavity, but this represents only a very small fraction of the absorbed cells (Courtice and Morris, 1953).

It is difficult to understand why little or no material is absorbed into the lymphatics on the pleural aspect of the diaphragm or the visceral pleura covering the lungs, for these membranes are subjected to considerable movement and stretching too. The structure of the basement membrane may be important since it is thin and fenestrated only over the intercostal spaces and in certain junctional regions of parietal and mediastinal pleura (Dybkowsky, 1866; Policard and Galy, 1941) The arrangement of the lymphatics may also be significant, since the vessels connecting the pleural lymphatics with those of the lungs have valves pointing towards the pleura (Miller) or have no definite valvular arrangement (Simer). Thus any stretching of the pulmonary subpleural plexus would be as likely to aspirate lymph from the lungs as to suck fluid in from the pleural cavity. In pulmonary oedema aspiration of fluid from the lung to the pleural cavity may occur (Graham, 1921), which suggests that the tendency for lymph to pass outwards is greater than the inward passage of pleural fluid. In the diaphragm, in the same way, the stretching of the lymphatics may aspirate lymph from the peritoneal lymphatics In the intercostal vessels, on the other hand, valves prevent the backward aspiration of lymph.

Rate of lymphatic absorption

Starling and Tubby (1894) injected solutions of carmine and methylene blue into the pleural sacs of dogs, and found that the urine became coloured before the thoracic duct lymph, indicating a predominance in blood absorption of these substances As with the peritoneal cavity, however, such experiments give no indication of the capacity of the lymphatics to remove naturally occurring effusions or exudates, for substances such as Starling and Tubby used will readily diffuse through the walls of blood capillaries. Pleural fluid, on the other hand, always contains protein, as does peritoneal fluid, and this protein will only be absorbed by the lymphatics. Yamada and his associates (1933) collected fluid by pleural puncture from the pleural sacs of several hundred healthy soldiers When the men had been quiet before puncture, fluid was obtained from 29 per cent of the subjects, but after severe work, it was secured in 70 per cent of the group. The amount of fluid varied from a drop to 20 g. and the protein concentration varied from 1·38 to 3 35 per cent. In normal animals, too, whenever pleural fluid can be collected the protein content is appreciable In pathological conditions, when considerable quantities of fluid may be present in the pleural cavities, the protein content is high. James (1949) found in man average concentrations of 2 1 per cent in heart failure, 4·2 per cent in carcinoma and 5·3 per cent in tuberculosis

The capacity of the lymphatics to absorb protein-rich fluids such as occur naturally has received little attention. Starling and Tubby observed

that blood serum was absorbed with " extreme slowness ", but they used only anaesthetized dogs and kept the experiment going only 2 to 3 hours. Courtice and Simmonds (1949*b*), however, found that in unanaesthetized rabbits a considerable amount of plasma, 6 ml./kg., was absorbed within 24 hours, while in rats the same quantity could be removed in 8 to 16 hours (Lake, Simmonds and Steinbeck, 1953). Although this rate of removal is much less than from the peritoneal cavity, it should not be

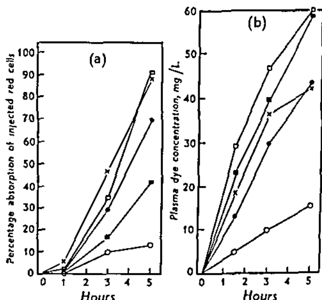


FIG 70—The effect of alterations in respiratory movement on the absorption of red cells labelled with P^{32} and of plasma protein labelled with T1824 from the pleural cavity of the rat

(Redrawn from Courtice and Morris, 1953)

regarded as slow. Red cells, too, are readily absorbed into the lymphatic vessels. Courtice and Morris (1953) injected 1 ml./kg. whole blood (homologous) with the cells labelled with P^{32} into the pleural cavities of the rat and found that 70 per cent of the injected cells were absorbed through the lymphatics into the blood stream in 5 hours.

As in the peritoneal cavity, lymphatic absorption is greatly affected by respiratory movement; nembutal anaesthesia slows while the inhalation of 5 per cent carbon dioxide considerably increases the rate of absorption of both labelled proteins and red cells from the pleural cavity, Fig. 70.

Diaphragmatic movement seems to play an important part in the absorption of various particles. Cooray (1949), using radio-opaque dyestuffs of various viscosities, studied the path of the material injected into the pleural cavity of the normal rat and of rats with the diaphragm paralysed. He found that loss of diaphragmatic movement caused by phrenic neurectomy, pneumothorax or pneumoperitoneum definitely delayed absorption. His criteria of retarded absorption were based on the visual record of a slowing in movement of the contrast agents towards the retro-cardiac mediastinal folds, which he regarded as the pleural absorbing surfaces. Courtice and Morris (1953) by quantitative methods found that phrenic neurectomy in the acute stage also caused a decreased absorption of red cells labelled with P^{32}. On the other hand, these authors showed clearly that labelled plasma protein was absorbed more rapidly than normal after the diaphragm had been acutely paralysed. This they attributed to the greatly increased absorption by the lymphatics of the parietal pleura When the phrenic nerves in rats were severed and the diaphragm paralysed, the intercostal and abdominal muscles very greatly increased their contractions in an endeavour to maintain an adequate pulmonary ventilation (Davis and Morris, 1953) Such an increased respiratory effort on the part of the intercostal muscles could increase absorption of protein even though absorption by the mediastinal route was decreased. The difference in the behaviour of the absorption of red cells and of plasma protein suggests that the red cells cannot so readily gain access to the lymphatics of the parietal pleura as can the much smaller protein molecules. After 2 or more weeks of diaphragmatic paralysis the absorption of both red cells and of plasma was approximately normal. A similar effect was seen in absorption from the peritoneal cavity. This can probably be explained by the gradually increased movements of the intercostal and abdominal muscles necessary to restore the correct intrapleural pressures. The restoration of lung movement, together with the tremendous exertion of the abdominal muscles, probably move the diaphragm passively and so increase the absorption of injected red cells through the mediastinal folds.

The Pericardial Cavity

The lymphatics of the parietal pericardium have not been extensively investigated. Drinker and Field (1931) showed that in the rabbit near the base of the heart where this membrane is heavily loaded with fat, lymphatics are abundant and drain into several small lymph nodes embedded in the basal cardiac tissue In the thin, transparent, pericardium towards the apex of the heart, however, no lymphatic vessels were seen, but it was pointed out that a few might be present as in the case of the thinnest parts of the omentum.

The visceral pericardium, or epicardium, on the other hand, contains

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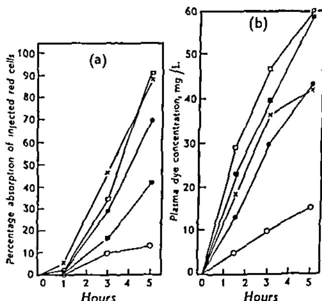


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pleural cavity Absorp-

nto pleural cavity

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As in the peritoneal cavity, lymphatic absorption is greatly affected by respiratory movement; nembutal anaesthesia slows while the inhalation of 5 per cent carbon dioxide considerably increases the rate of absorption of both labelled proteins and red cells from the pleural cavity, Fig. 70.

unite to form a single vessel which leaves the heart on the anterior surface of the pulmonary artery. Very few valves are found in any of the three capillary systems.

Patek found that when the lymphatics in the beating heart were filled with India ink, the ink was promptly washed away. This led him to think that lymph movement in the heart must be quite rapid. Of the mechanism of lymph movement in and from the heart he writes: "It seems probable that the pressure of the blood in the ventricle during diastole drives lymph from the subendocardial lymphatics into the myocardial lymphatics. During systole the contraction of the myocardium, as pointed out by Kampmeier, probably forces the lymph from the myocardial lymphatics into the subepicardial lymphatics and the pressure of the dilated heart against the pericardium toward the end of diastole may reasonably be expected to drive the lymph from the subepicardial lymphatics into the main lymphatic trunk leaving the heart."

Drinker, Warren, Maurer and McCarrell (1940) cannulated the lymph trunk at the base of the heart in dogs, and collected cardiac lymph for long periods of time. Table 27 shows the average flow of lymph from the

TABLE 27

Average flow of cardiac lymph in ten dogs

Weight *Kg*	*Heart Weight* *g*	*Blood Pressure* *mm Hg*	*Average lymph flow* *ml /hour*
8.0	77.0	142	0.31
11.8	111.0	129	0.46
11.8	94.0	94	0.52
11.8	91.0	130	0.56
14.6	114.5	112	0.59
11.0	85.0	160	0.63
14.0	102.0	104	0.95
14.4	91.0	120	0.95
11.0	97.5	140	1.63
11.5	91.5	112	1.65

From Drinker, Warren, Maurer and McCarrell (1940)

hearts of ten dogs. On an average the lymph flow was 0.8 ml per hour, but there was no correlation between dog weight, heart weight, blood pressure and lymph flow. The lymph always contained a high concentration of protein; in 18 dogs the range was 2.50 to 4.73 per cent with a mean of 3.69 per cent. This was somewhat higher than the protein level of pericardial fluid in those animals in which free fluid was found in the pericardial cavity. There is in the heart, therefore, a continual leakage of protein from the capillaries of the coronary circulation, and the rhythmic contractions of the cardiac muscle ensure its rapid removal by the lymphatic plexuses.

a plexus of lymphatic capillaries as well as larger collecting vessels which ultimately enter the lymph nodes at the base of the heart. Patek (1939) has shown that in the dog there are no collecting trunks in the endocardium or the myocardium, but the subendocardial and myocardial plexuses of lymphatic capillaries drain into the subepicardial vessels. After entering the lymph nodes the lymph emerges in a single trunk which may be cannulated and a mixture of lymph from all parts of the heart collected (Drinker *et al.* 1940). Valves are numerous in the larger subepicardial vessels but only a few have been found in the capillary plexuses.

When fluid was injected into the pericardial sac, lymphatic absorption occurred mainly at the base of the heart and not uniformly through the vessels of the pericardium (Drinker and Field, 1931). As in the peritoneal and pleural cavities, the naturally occurring fluid in the pericardial cavity contains a high concentration of protein. Maurer, Warren and Drinker (1940) found average protein values for normal pericardial fluid of 1·70 per cent in dogs, 2·16 per cent in rabbits, 1·71 per cent in monkeys and 2·42 per cent in cats, while in disease in man they found that the protein concentration was usually 3 to 4 per cent, but varied from 0·78 to 6·68 per cent. These fluids would therefore be removed by way of the lymphatics. Information concerning the rate of absorption of fluids from the pericardial cavity is, however, very scanty. Drinker and Field injected Ringer solution and serum into the pericardial cavity of rabbits. They found that when 3 to 7 ml. of Ringer solution was introduced, the average absorption in 1½ to 2 hours was 1·3 ml. per hour. With serum (rabbit or horse) they found that in four animals there was no absorption in 2 to 4 hours, while in two animals there was 100 per cent absorption. On the basis of these experiments the authors concluded that absorption of serum from the pericardial cavity of rabbits was negligible. It would seem, however, that further investigations are required to determine the capacity of the pericardial lymphatics to remove a protein-rich fluid from the pericardial cavity.

HEART

According to Patek (1939), the lymphatics of the heart are distributed as three plexuses, subendocardial, myocardial and subepicardial. The subendocardial vessels comprise capillaries which lie in a single plane. They drain into the myocardial plexus which is a profuse system of interconnected capillaries. There are no draining trunks in either the subendocardial or myocardial tissues. The lymph from these plexuses ultimately reaches the subepicardial network which covers the whole of each ventricle. The vessels in the subepicardial system unite to form drainage trunks, which accompany the coronary vessels and eventually

and adrenaline injection was accompanied by a lymph flow of 400 mg./min. or 24 ml./hr. It is, however, doubtful if a flow of lymph above 300 mg./min. could be maintained under the circulatory conditions that would exist in a dog during a long run. At the rate of 300 mg./min., the flow

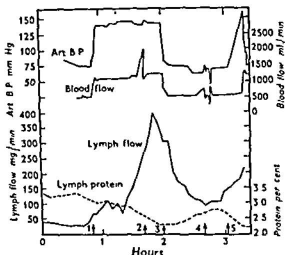


FIG 72—Starling heart-lung experiment with lymph collection

At arrow 1, cardiac inflow was increased and arterial resistance raised, at arrow 2, 2 5 ml of 1 50,000 adrenaline were placed in the venous reservoir, at arrow 3 cardiac inflow was decreased, at arrows 4 and 5, 1 000 ml of Ringer's solution were added to the blood, reducing the blood protein from 4 68 per cent to 1 54 per cent

(From Drinker, Warren, Maurer and McCarrell, 1940)

of cardiac lymph in a hound engaged in a twelve hours' chase would be approximately 18 ml. of lymph per hour, or 216 ml. for the twelve hours. In a dog, such as the one in the experiment cited, with a heart weighing 91 g., this would mean 2.4 ml of lymph per gram of heart during the twelve hours

JOINTS

The nature of the synovial tissue lining joint spaces has been widely debated. In reviewing the literature Bauer, Ropes and Waine (1940) conclude that the joint is a tissue space rather than a body cavity. The so-called synovial membrane conforms in structure to connective tissue with cell aggregations near the lumen, rather than a continuous lining membrane. In this synovial tissue are two plexuses of lymphatics, one near the lumen and one deeper. These lymphatics do not communicate directly with the cavity, but on the other hand, no barrier exists separating the articular cavity from the intercellular spaces of the synovial tissue.

The joint cavity normally contains varying amounts of synovial fluid.

When the work of the heart was increased by means of adrenaline or ephedrine, lymph flow at once became greater, and the effect continued as long as the drug acted. Fig. 71 shows such an experiment After a normal period of lymph collection of over an hour's duration, 130 mg of sodium nitrite were given intravenously. Lymph flow decreased slowly with the gradual fall in blood pressure. An intravenous injection of 13 mg of ephedrine sulphate raised the blood pressure and increased cardiac work, with an immediate augmentation of lymph flow. Lymph flow also increased, as it does in other tissues, when the tissue fluid formation was rapidly raised by acute plasmaphaeresis.

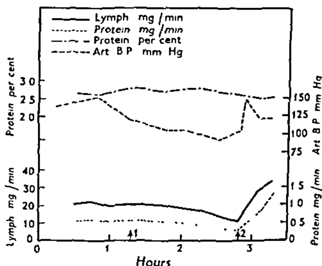


FIG 71 —Cardiac lymph flow, blood pressure, and percentage of lymph protein in a dog given sodium nitrite and ephedrine.

At arrow 1, 130 mg of sodium nitrite were given intravenously, at arrow 2, 13 mg of ephedrine sulphate (*From Drinker, Warren, Maurer and McCarrell, 1940*)

In order to obtain an idea of the possible volume of lymph flow from the heart under circulatory conditions simulating those of hard exercise, lymph was collected from the heart of a dog in a Starling heart-lung preparation. In this preparation, heparinized blood entered the superior vena cava, was driven by the right ventricle through the lungs and was delivered to an artificial peripheral circulation via the left subclavian artery. Under such circumstances, it is easy to increase the inflow of blood to the heart, the arterial resistance, and—by adding adrenaline to the venous reservoir—the vigour and completeness of each cardiac contraction. The conditions imposed are thus a crude representation of the cardiac activity accompanying heavy physical exercise. Fig. 72 is a chart of such an experiment. The dog weighed 11·3 kg.; the heart 91 g. The combination of increased inflow of blood to the heart, increased blood pressure

and adrenaline injection was accompanied by a lymph flow of 400 mg./min. or 24 ml./hr. It is, however, doubtful if a flow of lymph above 300 mg./min. could be maintained under the circulatory conditions that would exist in a dog during a long run. At the rate of 300 mg./min., the flow

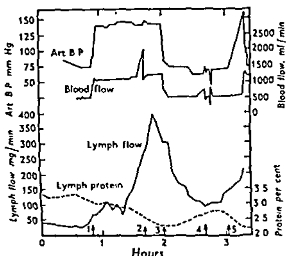


FIG 72.—Starling heart-lung experiment with lymph collection

At arrow 1 cardiac inflow was increased and arterial resistance raised, at arrow 2 2.5 ml of 1 : 50,000 adrenaline were placed in the venous reservoir, at arrow 3 cardiac inflow was decreased, at arrows 4 and 5, 1,000 ml. of Ringer's solution were added to the blood, reducing the blood protein from 4.68 per cent to 1.54 per cent

(From Drinker, Warren, Maurer and McCarrell, 1940)

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The joint cavity normally contains varying amounts of synovial fluid.

From the chemical analyses of joint fluid obtained from cattle, Ropes, Bennett and Bauer (1939) concluded that synovial fluid is a dialysate of blood plasma to which mucin has in some way been added. On an average they found that the total protein concentration of this fluid was 1·02 g. per cent, of which 0·71 was albumin, 0·17 globulin and 0·14 mucin. One of the several theories postulated for the formation of synovial fluid is that this fluid is the liquid matrix of the connective tissue lining an enlarged tissue space, the mucin corresponding to the mucoid constituent of other connective tissues. A similar concept is that the synovial fluid is a dialysate of blood plasma to which mucin is added as the fluid diffuses through the connective tissue surrounding the joint cavity.

If we accept this concept, we would expect that the exchange of substances between the blood and synovial fluid and the rôle of the lymphatics are the same as in any connective tissue fluid. There is in fact a fairly rapid exchange of materials of small molecular size between the blood and the synovial fluid. For example thiocyanate and glucose readily diffuse into the joint cavities when injected intravenously (cf. Bauer, Ropes and Waine, 1940) and mecholyl is readily absorbed directly into the blood stream when injected into a joint cavity (Rhinelander, Bennett and Bauer, 1939). Large protein molecules introduced into the joint cavity, on the other hand, are absorbed only into the lymphatics. They presumably diffuse between the cells of the synovial tissue to enter the underlying lymphatics. Bauer, Short and Bennett (1933) showed that egg white and horse serum albumin readily entered the lymphatics and that passive movement of the joint greatly accelerated their removal. Horse serum globulin, on the other hand, did not leave the joints in these experiments. We may regard synovial fluid, therefore, as a somewhat specialized tissue fluid, which nourishes the articular cartilages As in other regions, there is an extravascular circulation of protein in which the lymphatics play an essential rôle. In pathological lesions of joints, therefore, when the protein content of the synovial fluid approaches that of the plasma (Allison *et al.*, 1926) we should expect the lymphatics to play a major part in the resolution of the excess fluid.

When particulate matter, such as India ink or graphite, is introduced into a joint cavity, the particles slowly enter the lymphatics, sometimes apparently carried by phagocytes, and at other times without such assistance ; but in any event removal is extremely slow (Key, 1926 ; Rynearson, 1931). It would be interesting, in view of the work done on the serous cavities, to investigate the removal of particles such as red blood cells which may be present in the synovial fluid in large numbers after trauma. Maybe they would gain access to the lymphatics without first undergoing phagocytosis, aided by movement of the joint.

Spleen

The exact structure of the walls of the venous sinuses of the spleen is still debatable. One view is that the sinus wall is a network of longitudinal, rod-shaped fixed macrophages and circular reticular fibres and that this wall is therefore perforated by many permanent openings, or latticed Another view is that these sinuses are not open structures in this latticed sense, but that their walls are extremely permeable to blood plasma, thus permitting them to store cells in large numbers (Knisely, 1936). Whatever view is correct, it is apparent that the vessels of the spleen are very permeable to the plasma proteins.

Lymphatics have, in general, not been found in the stroma of the spleen, but are present in the capsule and in the thickest trabeculae. Snook (1946), however, has described lymphatic vessels accompanying the arteries in the white pulp of the spleen in certain animals, the guinea-pig, mole, mouse and horse. In the absence of lymphatics in the red pulp, the fluid that passes through the walls of the capillaries and sinuses must permeate the splenic stroma before reaching the lymphatics in the trabeculae and capsule. In this respect the formation of lymph in the spleen resembles that in the liver. Recently, Hatta, Okada, Morita and Mishima (1955) have collected splenic lymph in dogs under methane anaesthesia. In seven dogs of 7 to 13 kg. body weight and 31 to 68 g. splenic weight, the lymph flow varied from 0 12 to 0 54 ml /hr , and the average protein concentration of the lymph was 61 per cent of that in the plasma.

When the splenic veins are ligated in the dog to produce venous congestion, the escape of fluid from the splenic vessels increases and some red cells also escape so that the lymph entering the cisterna chyli from the spleen may be seen to be blood-stained (Barcroft and Florey, 1928). Trypan blue injected into the splenic artery was also soon observed in the cisterna chyli. These experiments show that the fluid escaping from the splenic vessels rapidly finds its way into the lymphatics.

Genito-Urinary System

Kidneys

There is little direct information concerning the formation, amount and composition of lymph from the urinary tract Clinicians have been interested in the spread of infections and of malignant disease, and the gross lines of lymph drainage are fairly well understood , but there is comparatively little material dealing with the finer aspects of lymphatic function in this area

There is a profuse network of lymphatics in the capsule of the kidney,

and a rich supply in the parenchyma (cf. Poirier, Cuneo and Delamere, 1904, Bartels, 1909; Kumita, 1909; Rouvière, 1932; Abeshouse, 1934). The capsular vessels drain into collecting trunks which either perforate the fibrous capsule to communicate with the lymphatics of the perirenal fat capsule or connect with the deep collecting trunks of the parenchyma The evidence suggests that the parenchymal lymphatics surround both the glomeruli and tubules, but are not present within the glomeruli Those vessels surrounding the glomeruli and the tubules in the cortical zone may communicate with the plexus situated beneath the fibrous capsule *In the medullary zone the lymph capillaries surround* the loops of Henle and the collecting tubules, and give rise to larger vessels which pass up to the base of the pyramids to unite with trunks from the cortical network. The so-formed larger collecting ducts accompany the arteries and veins and finally reach the hilum, where they pass along with the other elements of the pedicle to the juxta-aortic and pre-aortic lymph glands. Peirce (1944), however, was unable to demonstrate the presence of a lymphatic network about Bowman's capsule or lying in the medullary rays.

Schmidt and Hayman (1929–1930) showed, in the dog, that fluid steadily leaves the kidney by lymphatics, in addition to that going out by the ureter; and that diuretics augment both lymph and urine formation. They measured the flow in the thoracic duct and in the abdominal receptaculum of dogs after preliminary evisceration, unilateral nephrectomy, ligation of aorta and inferior vena cava below the remaining kidney, *and ligation of the portal vein and hepatic artery.*

More recently, Sugarman, Friedman, Barrett and Addis (1942) and Kaplan, Friedman and Kruger (1943) cannulated both capsular and hilar lymphatic vessels of the kidney of dogs, other visible ducts being ligated. When they injected the dye, T1824, into the medullary region it readily appeared in the hilar lymph vessels but not in those of the capsule, when the dye was injected into the cortical area it often appeared in both the capsular and hilar vessels. These experiments confirmed the anatomical connexions of the cortical and medullary lymphatics already mentioned. In several experiments these authors measured the flow and composition of the renal lymph The mean values and ranges in their experiments are given in Table 28

From this table it is seen that the average amount of glomerular filtrate formed per hour was on an average 3,456 ml. of which 75 ml. passed into the urine and 3,381 ml. was reabsorbed. Compared with this large volume of reabsorbed fluid the cannulated lymphatic drained only 1 4 ml. Even if we assume that the total lymph flow from the kidney is ten times this, since in some kidneys up to 10 efferent vessels were ligated, the volume of fluid drained by the lymphatics is but a small fraction of the fluid

TABLE 28

Flow and composition of renal lymph in the dog

Flow from single cannulated trunk (11) 0·0232 g./min. (0·0054–0·0550), i.e. 1·4 ml./hr.

Protein concentration (11)	Lymph	1·84 g. %	(0·56–4·21)
	Renal artery	5·81 g. %	(5·18–6·88)
	Renal vein	5·80 g. %	(5·18–6·60)
Urea concentration (11)	Lymph	69·7 mg. %	(38·7–164·0)
	Renal artery	53·1 mg. %	(22·8–154·6)
	Renal vein	51·1 mg. %	(22·8–151·2)
Glucose concentration (9)	Plasma	103·5 mg. %	(70–135)
	Renal lymph	92·7 mg. %	(70–115)
	Cervical lymph	101·9 mg. %	(84–125)
Inulin concentration during I.V. infusions of inulin	Plasma	147·6 mg. %	5 expts
	Cervical duct	138·8 mg. %	
	Plasma	121·2 mg. %	8 expts
	Renal lymph	82·5 mg. %	
	Inulin clearance	57·6 ml./min.	8 expts
	Urine volume	1·25 ml./min.	

Modified from Sugarman *et al.* (1942) and Kaplan *et al.* (1943)

filtered and reabsorbed. It is evident, therefore, that in these experiments the lymphatics played little part in tubular reabsorption. The higher urea concentration in the renal lymph and the lower inulin concentration (obtained during the infusion of inulin to ascertain the inulin clearance) [illegible]

the renal lymphatics is a mixture of tissue fluid from various regions of the kidney, and that this tissue fluid is formed in part from capillary filtrate and in part from reabsorbed tubular fluid. It is quite probable that the composition of tissue fluid and so of lymph varies considerably in different regions of the kidney.

Chyluria. Although the renal lymphatics play little part in normal tubular reabsorption, on rare occasions thoracic duct chyle may enter the urine through a fistulous communication between the lymphatic and urinary systems. This is usually caused by lymphatic obstruction of the thoracic duct by the filaria parasite or other pathological states, so that the intestinal chyle is forced from the lacteals in a retrograde direction towards the pelvis of the kidney, thence through lymphatics of the abdominal wall to anastomose with vessels of the upper half of the body. In this collateral lymphatic circulation one or more of the vessels may rupture into the pelvis of the kidney, into the ureter or into the bladder, giving rise to chyluria (cf. Abeshouse, 1934).

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ascribable to lymphatic obstruction. In this condition the lymphatics of testis and scrotum maintain their separate paths (Paul, 1950), so the testis rarely becomes involved. The lack of anastomosis between the lymphatics of testicle and scrotum has also been shown by Barringer and Earl (1941) who found no metastases in the inguinal glands in 37 cases of teratoma of the testis until the growth had invaded the scrotal integuments.

Ovaries

The stroma of the ovary contains a network of valved lymphatic vessels which are often associated with the arteries as periarterial lymphatics. These vessels drain into collecting vessels at the hilum. Andersen (1926) showed that in the sow the lymph vessels vary greatly in size in the different stages of the oestrous cycle. The very youngest ovarian follicles have no special vascular or lymphatic system of their own, but lie scattered among the vessels of the stroma As a follicle develops, it acquires a wreath of blood-capillaries, following which the growth of a wreath of lymphatic vessels is observed A complete network of many-valved channels develops in the theca externa and a little later another complete wreath of lymphatics between the theca interna and granulosa may be seen

When the ovum is extruded, the follicle collapses and corrugations of the theca interna and granulosa are produced No new lymphatics have been observed growing into the developing corpus luteum during the first one or two days after ovulation. After this time, however, lymph vessels grow into tongues of lutein tissue usually associated with the arterioles as periarteriolar plexuses. At the line where these tongues of growing tissue meet, there develops a central network of lymphatic vessels. In the mature corpus luteum there is, therefore, a central lymphatic sinus with many branches, apparently arising from the subgranulosal network, and alternating with its branches are smaller sinuses connected more directly with the peripheral network. These two sets of lymphatics, the central and the peripheral, are connected by fine capillaries

As the corpus luteum regresses, the lymphatics are among the first structures to degenerate and ultimately they disappear entirely. When the next corpus luteum is mature, no vessels can be injected in the previous one

No lymph has been collected from the vessels of the ovary during the various phases of the oestrous cycle, but Andersen suggests that the lymphatics may be the pathway of drainage of the lutein secretory products

Fallopian Tubes

The general pattern of the lymphatics of the Fallopian tubes is the same throughout and consists of a subserous and mucosal plexus Lymph

Ureters and Bladder

The ureters contain a well-developed network of lymph capillaries in the mucosa and in the muscular coat (Parker, 1940). The lymphatics of the bladder form a complicated net of capillaries in the muscular coat and in the connective tissue just outside it. Attempts to inject lymphatics in the mucosa of the bladder have failed (Powell, 1944). The lymphatic network begins in the submucosa from which small ducts pass to the external surface to join collecting ducts in the adventitia covering the bladder. As the urinary tract descends from the kidney to the bladder the lymphatics become further from the epithelial surface, and lymphatic absorption of substances penetrating the epithelial surface becomes increasingly difficult. Nothing is known, however, of the formation and character of the lymph from the ureters or bladder.

Penis and Urethra

The skin of the penis contains dense meshworks of lymphatics, similar to those found elsewhere in the skin. The urethral mucosa is also heavily supplied with lymphatics; but no one has measured the volume or composition of lymph from these regions.

Scrotum and Testicles

The scrotal skin is profusely supplied with lymph capillaries; and it is of interest that it also contains muscle, which by its contraction causes lymph to move along draining trunks. This lymph drains into the inguinal lymph nodes. In the testis networks of lymphatic capillaries can be demonstrated between the seminiferous tubules. These vessels drain into collecting ducts which ascend in the spermatic cord to reach the aortic lymph nodes. Allen (1943) has also outlined in man, but not in other animals, a plexus of lymph capillaries in the parietal part of the tunica vaginalis comparable to the subserous plexuses of the diaphragm and intercostal pleura. This plexus joins with the collecting ducts draining the testis. There are no anastomotic connections between the lymphatics of the scrotum and those of the testis

In man there is a closed serous cavity surrounding the testis, while in animals this cavity communicates with the peritoneal cavity. Normally no fluid can be collected from it In 32 cases of hydrocele, however, Vecchi (1912) reported the protein content as varying between 2·43 and 9·51 per cent. Since inflammation undoubtedly complicated these findings, they give us little indication of the normal composition of fluid lubricating the serous surfaces about the testicle. Nor do we know anything of the volume of lymph coming from the testis, under either normal or abnormal conditions.

The scrotum may become the seat of elephantiatic change, a condition

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from these two plexuses drains into lymph channels in the mesosalpinx by a common set of valved collecting vessels in the connective tissue between the longitudinal and circular muscle layers (Andersen, 1927). It has been suggested that the anatomical arrangement of the mucosa and of the rich mucosal plexus of lymphatics provides a wide area for absorption. There are, however, no experiments which show to what extent substances from the peritoneal cavity or from the uterus may be absorbed from the mucosa of the Fallopian tubes into the underlying lymphatics.

Uterus, Vagina and Vulvar region

Wislocki and Dempsey (1939) injected the lymphatics of the uterus, vagina and sexual skin of rhesus monkeys with a suspension of India ink In the walls of the vagina there is a rich network of lymphatics extending *from beneath the surface epithelium, throughout the tunica propria* into the muscular and outer fibrous coats where collecting trunks are formed In the sexually mature animal the vessels are much larger, though no more abundant than in the juvenile.

Injections in the uterus show that the endometrium contains lymph vessels which form characteristic arcades occupying only the deeper three-quarters of the mucosa. At the junction of the mucosa and muscularis is a dense plexus of lymph vessels which drain into the rich intrinsic plexuses of the myometrium During pregnancy these plexuses hypertrophy, and although probably not numerically increased the lymphatic vessels are tremendously enlarged in calibre.

In the sexual skin of the monkey is a very dense network of vessels of relatively small calibre situated in the corium. From this plexus lymph is drained into larger channels which run in the subjacent subcutaneous tissue

The function of the rich lymphatic networks throughout the female genital tract is believed to be the removal of the physiological oedema or hydration to which these tissues are subjected under the influence of the sex hormones. Aykroyd and Zuckerman (1938–1939), for example, found that the oedema fluid of the swollen sexual skin at oestrus had a protein content of 2 58 per cent A rich network of lymphatics would be necessary to remove this fluid and so allow the tissues to return to their resting condition

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that in the mammal. Isayama, for example, estimated that an amount of fluid equal to fifty times the blood volume of the frog passes out of the blood and becomes lymph every twenty-four hours. Conklin found that, though variable and depending on the circulatory state of the animal at the time, the volume of lymph formed per day was many times the total blood volume. Due to the capacious lymph spaces and the pulsating hearts this large volume of fluid, which contained all fractions of the plasma proteins in fairly high concentration, could be continuously transferred from the blood to the lymph and back to the blood again. When the lymph hearts were destroyed or were paralysed by prolonged curarization, oedema and death resulted

Although the lymph hearts are essential in the frog for the rapid lymph circulation, other factors, such as muscle movement and the massaging effect of the pulsation of blood vessels, are also important. If one fills the lymphatic capillaries in the web with a graphite solution, the particles floating in the fluid begin to move out of the web lymphatics as soon as the injection pressure is released. There are two blood capillary plexuses in the web, with the lymphatic plexus between them and thus in an ideal position to be affected by the vigorous pulsation of the small web arteries and capillaries

The frog has no lymph nodes, nor do we see nodes until we reach certain diving birds. Scattered collections of lymphocytes are present in frogs and snakes but there are no organized lymph filters to retard lymph flow, or to add to the lymphatic system other functions than the simple one of circulating fluid which has become extravascular

Lymph Flow in the Mammal

The lymphatic system in the mammal possesses no lymph hearts similar to those in amphibia What intrinsic mechanism remains is seen, in some animals and in some circumstances, as rhythmic contraction of the large collecting lymph ducts This contractility of the lymph vessels has been described in Chapter 1. The balance of the evidence suggests that this intrinsic mechanism plays only a minor rôle in lymph propulsion in mammals Therefore, compared with the frog, the lymph flow in general is very sluggish As we have seen in Chapters 2 and 3, the total lymph flow is normally less than the total blood volume per day ; so that whereas in the frog interruption of the lymph flow by destruction or paralysis of the lymph hearts rapidly causes death, the effects of lymphatic blockage in the mammal are somewhat more gradual In the dog, for example, blockage of the lymphatics of a limb gives rise to elephantiasis over a relatively prolonged period (Drinker, Field and Homans, 1934), but a complete blockage of all lymph return, which is extremely difficult

CHAPTER 4

LYMPH FLOW, LYMPH PRESSURE AND LYMPH COMPOSITION

The flow of lymph from a region may be raised, for a time at least, by increasing the mechanical forces responsible for lymph propulsion along the lymphatic vessels. For example, pressure on the abdominal wall will force lymph from the cisterna chyli along the thoracic duct with a resultant sudden increase in flow. We have seen that lymph flow will also be increased when the capillary filtration rate is increased either by raising the filtration pressure or by increasing capillary permeability. In considering lymph flow, therefore, we must be careful to distinguish between changes in the forces of lymph propulsion and changes in lymph production.

Lymph Flow in Amphibia

In Chapter 1 we have mentioned that the primary lymph sacs of some animals are converted to lymph hearts which by their rhythmic contractility propel the lymph back to the blood stream. These lymph hearts are best developed in amphibia and their physiological function has been studied mainly in the frog (Conklin, 1930*a*, *b*, *c*; Isayama, 1924–1925*a* and *b*). In this animal, tissue fluid, which is made up of capillary filtrate and of water absorbed through the skin, enters the lymph capillaries, passes into the larger lymphatic vessels and then into the lymph sacs or spaces. This lymph then enters the afferent pores of the lymph hearts and is pumped through the efferent pores into the veins. The anterior pair of lymph hearts lie on the dorsal surface of the transverse processes of the third vertebra and communicate by their efferent pores with the anterior vertebral vein. The posterior pair of lymph hearts are more superficial, just lateral to the urostyle, and they communicate by their efferent pores with the transverse iliac veins. The pulsation of all four hearts is not synchronous, but the two hearts on either side beat in unison. Each heart possesses a spinal centre and intraspinal connexions exist between the anterior and posterior hearts of the same side (Pratt and Reid, 1939; Enderle, 1950–1951). The rate is very variable, but in the majority of cases is about 50 to 60 per minute.

The normal outflow of fluid from the blood vessels and its return to the blood through the lymphatic apparatus is very great compared with

Although these experiments show that in a resting or immobilized tissue, the lymph flow is virtually nil, McMaster (1941–1942) has expressed the belief that there is lymph flow from a quiescent part. In experiments

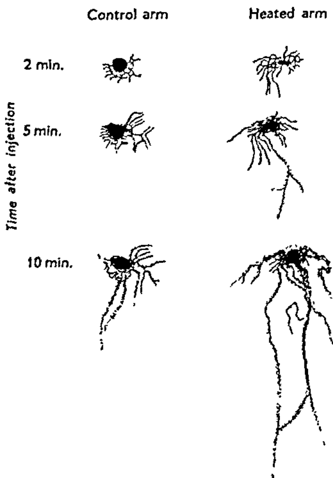


FIG 73.—Tracings of the movement of dye along the lymphatic vessels of the forearm after intradermal injection of the vital dye, patent blue V.

The diagram in the first column shows the result in the *normal resting arm* and in the second column the effect of immersing the resting arm in warm water at 46–47° C. In the warmed arm the blue "streamers" developed more rapidly. Active movement of the arm very greatly accelerated the formation of "streamers" by increasing the flow of lymph in the lymphatics of the skin.

(From McMaster, 1937, by kind permission of the author and the *Journal of Experimental Medicine*)

on the mouse's ear, Hudack and McMaster (1932) outlined some of the lymphatics by injection of a small amount of dye. They observed that a stream of dye-containing lymph was displaced, diluted and swept

to achieve, will lead to death in a much shorter time (Blalock, Robinson, Cunningham and Gray, 1937).

Movement of lymph from the periphery to the centre is effected by pressure gradients brought about mainly by forces outside the lymphatic system McMaster (1947) found that in the skin of the mouse's ear the tissue *pressure just outside a lymph capillary was normally higher than* the pressure in the lumen of the vessel. On an average the tissue pressure was 1·9 cm water while the pressure in the lumen of the capillary was 1·2 cm. water. Tissue fluid, therefore, enters lymphatic capillaries because of this gradient of pressure. It seems reasonable to suppose that once inside a capillary the retention of fluid is due to the higher pressure of the interstitial tissue fluid outside. We have shown in Chapter 3, however, that in certain conditions lymph may leak out of the lymph vessels to form free fluid in the pleural or peritoneal cavities Presumably in these cases the pressure inside the lymph vessels of the pleura or of the liver is greater than that of the surrounding tissues.

Propulsion of lymph from one region to another can only be brought about by some extrinsic forces raising the tissue pressure. The lymph pressure in the vessels will, therefore, rise and the lymph will move centripetally to a region where the pressure is lower. A necessary adjunct to this extrinsic method of lymph propulsion is the provision of numerous valves which prevent the centrifugal movement of lymph from any region Lymph can, therefore, move only in the direction determined by the anatomical disposition of the valves.

When a tissue is at complete rest, the interstitial tension and the pressure within the lymphatic vessels are nearly the same. Under these conditions there is usually very little flow along the lymphatics. For example, when the lymphatics are observed in the ear-chamber of the rabbit movement of fluid is extremely slight. Floating cells in the lymph may be watched for hours without progressing. In such a preparation the lack of movement of the ear would reduce the propulsion of lymph almost to zero These observations led Clark (1936) to doubt whether these lymphatics played any useful rôle whatever. The movement of lymph in the vessels of a normal ear of a rabbit, would, however, almost certainly be very different from that in the vessels of a chamber.

The sluggishness of the lymph flow may also be seen when a cannula is inserted into a leg duct of an anaesthetized animal. No lymph as a rule flows into the cannula. Tissue fluid enters the lymph vessels and reaches an equilibrium in which lymph pressure and tissue tension must be about the same. On raising the tissue tension by massage or passive movement the lymph is propelled along into the cannula, the intralymphatic pressure then falls slightly and the vessels again fill. (For the cervical duct, see Chapter 6.)

the passive movement of the head of dogs in the collection of cervical duct lymph.

The volume of lymph obtained by this method also probably does not give an accurate assessment of lymph flow in an unanaesthetized animal. In experiments, however, where relative changes in lymph flow due to different conditions are being determined, these devices for uniform passive movement are likely to give more consistent results than periodic massage.

The flow of lymph from the limb ducts or cervical ducts, using either massage or passive movement, is not very great. With passive movement of the foot, the lymph flow from the duct cannulated by Haynes was 0·1 to 0·7 ml./hr., whereas Courtice (1951) in a group of 17 dogs obtained by periodic massage from one of the lymph ducts of the hind leg just above the ankle, an average flow of 1·1 ml./hr. With the cervical ducts McCarrell (1940*b*) in a series of 18 dogs and using passive motion found an average lymph flow of 2·2 ml./hr., whereas Courtice (1951) obtained an average flow of 1·1 ml./hr. from one cervical duct in 18 dogs.

Muscular activity. The effect of muscle contraction in raising tissue tension has been described in Chapter 2. The height of this increase will depend on the degree of muscle contraction and on the anatomical disposition of fascial planes. The resultant changes in lymph flow may be observed in lymph collected from different parts of the body.

Muscular activity and lymph flow from a limb. White, Field and Drinker (1933) cannulated lymphatics in the fore and hind legs of dogs, using only local anaesthesia. With the cannula in place, the lymph flow may be followed while the animal rests, walks or runs. A typical experiment, taken from their paper, is as follows:

Dog 2—March 15, 1932. Standing, walking and running

9 40–10 20	Dog lying quietly on table. Left fore paw lymphatic cannulated under novocaine
10 20	Lymph protein 1·98 per cent
10 50	Lymph protein 2·04 per cent. There was no spontaneous flow of lymph. Specimens were obtained by light massage of the foot.
11 01–11 12	Effect of walking (see Table 29)
11 12–11 30	Lymph protein 1·61 per cent. Lying quietly.
11 31–11 38	Effect of running (see Table 30).
11 55	Dog fed 0·5 kg. lean meat
11 55–12 45	Dog resting quietly
12 45	Lymph protein 2·12 per cent
12 45–1 45	Dog resting. Occasional brief periods of motion
1 00	Lymph protein 1·90 per cent
1.45	Lymph protein 1·48 per cent

away by another stream, itself unseen, draining from tissue remote from the region of injection. Coming from a normal, uninjected region of the ear, this clear lymph could not have been propelled by the pressure of injection. In the skin of the forearm of man, Hudack and McMaster (1933) and McMaster (1937) similarly showed some movement of lymph The intracutaneous injection of very small volumes (0·01–0 02 ml) of dye solution led to "*streamer*" *formation as the dye moved along the* lymphatics, Fig 73 Although this "streamer" formation showed that at rest there was in these experiments some lymph movement, the volume of flow was probably extremely small. The point of chief interest, however, is the fact that substances entering a lymphatic through a wound can be carried far in a short time. When the tissue tension was raised by warming the arm or by muscle movement, the flow, assessed by "streamer" formation, was greatly increased

Extrinsic factors affecting lymph flow

Massage. In experiments on lymph formation, one of the difficulties is to get a measure of lymph flow from relatively quiescent tissues If the cervical lymphatic trunks in the anaesthetized dog are cannulated, no lymph will flow spontaneously, but one can obtain a flow of lymph all day long by massaging the neck above the cannulae along the line of the deep cervical ducts This massage empties the collecting vessels, which in due course fill again, when they can be emptied once more by massage Lymph obtained in this way has been very useful for chemical studies It is difficult to assess, however, how the volume flow so obtained would compare with the normal flow in an unanaesthetized animal. No doubt this latter would vary enormously throughout the day with the degree of activity of the individual. In a similar way, the lymph flow from the thoracic duct, although spontaneous, may for a time be greatly increased by pressure on or by massage of the abdominal wall over the cisterna chyli

Passive motion. Passive movement of joints will vary the tissue pressure and so will propel lymph along lymph channels. Various mechanical devices have been used to produce a uniform, rhythmical passive motion of either a limb or of the head and neck. Haynes (1932*a*) cannulated lymphatics in the ankle of the dog, and attached the foot to a rotating crank, so that flexion and extension of the foot and ankle occurred at a rate of 40 times a minute This produced a slight but uniform flow of lymph, sufficient for a number of experiments upon the effects on lymph flow of haemorrhage, venous pressure, etc., but not yielding enough lymph for chemical analysis unless very long collection periods were employed. A similar device has been used by McCarrell (1939*a*) for

tissue fluid are correspondingly more concentrated. The effect of rest in the unanaesthetized animal illustrates the readiness with which protein concentration in the lymph may be increased. In the protocol cited, at 11.35, following four minutes' running, the lymph protein was 1·42 per cent. At the end of his run, the animal was fed and then rested fifty minutes. Lymph obtained by light massage at 12.45 contained 2·12 per cent of protein.

The effect of muscular activity in unanaesthetized dogs has also been determined on the lymph flow from the thoracic duct and from the liver. Here the flow is normally spontaneous, and, as we have seen in the last chapter, quite considerable; but mild exercise, walking at 40 yards per minute, increased the thoracic duct flow by 270 per cent and the liver lymph flow by 80 per cent. This increase was observed during only a brief experimental period and probably represented the effect of increased tissue pressure in emptying the lymphatics rather than the actual increase in lymph formation.

The effect of respiration on lymph flow. While most muscle activity is sporadic, and the effect on lymph flow is therefore very variable from time to time during the course of a day, respiratory movement provides a continuous, rhythmic pumping action which has a considerable effect on lymph propulsion from the thoracic and abdominal cavities. As a result of this, we find that although at rest there is generally no spontaneous lymph flow from the limb and cervical ducts, lymph will flow into cannulae in the thoracic or right lymph duct.

Respiratory movements may influence the propulsion of lymph in different ways in different lymph vessels. The phasic changes in intrapleural pressure are probably important in propelling lymph in thoracic duct and mediastinal lymphatics through the thoracic cavity. Meltzer (1892) showed that intrapleural pressure changes were transmitted to the tissues of the lower mediastinum but not to the upper mediastinum. Fry *et al* (1952) and others have shown that intrapleural pressure changes may be registered within the oesophagus. The phasic variations in intrapleural pressure should, therefore, provide a pumping mechanism propelling lymph in valved lymphatics from lower to upper mediastinum. The amount pumped varies, presumably, with the amplitude and frequency of pressure changes. For example, increased respiratory movement brought about by inspiring 5 per cent carbon dioxide increases the amplitude of the intrapleural pressure variations and at the same time increases lymphatic absorption from the pleural cavity (Davis and Morris, 1953; Courtice and Morris, 1953). In everyday life, too, sudden large pressure changes, such as occur in coughing for example, are superimposed on the smaller and regular variations observed at rest.

The extent to which pressures measured between inner and outer

TABLE 29

Flow and protein concentration of lymph from leg duct of the dog during walking

Time	*Distance travelled metres*	*Lymph flow ml.*	*Protein concentration %*
11.01			
11 02	25	0 08	1·98
11.03	100	0 12	
11 04	100	0 06	
11.05	100	0 06	
11 06	100	0 05	1 54
11.07	100	0 04	
11 09	100	0 04	1·52
11 10	100	0 04	
11 11	100	0 04	
11 12	100	0 04	1·52

From White, Field and Drinker (1933)

TABLE 30

Flow and protein concentration of lymph from leg duct of the dog during running

Time	*Distance travelled metres*	*Lymph flow ml.*	*Protein concentration %*
11 31			
11 32	200	0 01	
11 33	200	0 07	1·58
11 34	200	0 05	
11 35	200	0 05	1 42
11 36	200	0 05	
11 37	200	0 05	
11 38	200	0 05	

From White, Field and Drinker (1933)

These experiments show that whereas at rest there was no significant lymph flow, on walking or running the flow from the cannulated duct was appreciable. In the experiment quoted the flow was approximately 0·04 ml /min. while walking 100 metres per minute and 0·05 ml /min. while running 200 metres per minute. Under these conditions, then, the lymph flow was 2 to 3 ml. per hour from one cannulated vessel. During uniform activity the lymph flow soon became remarkably constant. As the flow became uniform, so did the concentration of lymph protein. When constancy of protein was reached in 8 dogs, the concentrations were from 1 52 to 0·5 per cent with an average of 0·99 per cent. These are lower figures than those obtained in anaesthetized dogs, where lymph from the foot is made to flow by means of passive motion or massage. In the anaesthetized animal conditions are better for reabsorption than in the walking animal, and the lymph and presumably the

when pressure proximal to the segment falls. It is quite possible that the onward movement of lymph may be intermittent within the thorax and the outflow into the veins continuous or synchronous with a different phase of respiration. It seems, then, that respiratory movement plays an essential part in the constant return of lymph along the main lymph channels from the abdominal and thoracic cavities. This extrinsic mechanism more or less replaces the intrinsic mechanism in the amphibia. In addition to this respiratory pump, sudden large changes of pressure in the thoracic or abdominal cavity must also play a part in emptying the lymph channels.

The conditions within the lung tissue are in some way different from those in the walls of the thoracic and abdominal cavities With normal respiration the pressure within the alveoli varies but little, and if this pressure is transmitted to the nearby lung tissue, there will be very little pressure variation with the different respiratory phases. In Chapter 3 the sluggishness of the lymph flow from the lungs has been fully discussed.

Pulsation of blood vessels. We have described in Chapter 3 how cardiac activity affects lymph flow from the heart itself. The pulsations of the blood vessels, by their massaging effect on nearby lymph vessels, also have an effect on lymph flow. Parsons and McMaster (1938) reported experiments on the ear of the rabbit in which they assessed lymph flow by the spread of injected dye along lymphatics. When the ear was perfused under constant pressure, there was almost no lymph flow, whereas under pulsatile pressure lymph flow was far more rapid In thin tissues, such as the ear of the mouse, the web of the frog and the mammalian lung, vascular pulsation probably produces a slight normal flow of lymph Cressman and Blalock (1939) have called attention to the fact that the receptaculum chyli is so placed as to receive pulsations from the aorta. More recently Webb and Starzl (1953) have recorded the pulse waves in the thoracic duct cannulated just above the diaphragm and at the root of the neck. The intralymphatic pulsations were synchronous with those of adjacent arteries, and in the neck also with the venous pulses, while the systolic-diastolic variation in pressure amounted to about 20 to 30 mm water, e g 70 systolic and 40 diastolic.

While there seems no doubt that vascular pulsations cause some lymph movement, this is extremely small when compared with the other effects discussed above.

Peristalsis and villous movement. The probable effects of villous movement on the [illegible]

ideal conditions for lymph flow during peristaltic activity. Watkins and

pleural layers are transmitted to efferent lymph trunks, such as the retrosternal vessels is unknown. Such vessels might be affected more directly by changes in tissue pressure or by traction and relaxation of connective tissue attachments, associated with movements of the chest wall. Intrapleural pressure changes might give little indication of pumping pressures developed in this way. Stretching and relaxation of mediastinal folds could affect mediastinal efferent vessels in a similar way. These effects on lymph drainage have been shown in experiments where the diaphragm was paralysed by phrenic avulsion in rats. Absorption of fluid into the lymphatics from the peritoneal cavity and lower mediastinum was decreased, because of the diminished movement of the diaphragm; but, *even though the respiratory fluctuations of intrapleural pressure were* small, the flow in the intercostal region was increased by the much greater movement of the intercostal muscles (Morris, 1953; Courtice and Morris, 1953).

Respiratory movements also affect the venous pressure against which the thoracic and right lymph ducts empty their contents. It seems that here the only measurements of the pressure gradient are those of Rouvière and Valette (1937). They measured the side pressure in the thoracic duct in dogs without obstructing the flow, together with the venous pressure near the point of lymphatic entry. The thoracic duct pressure was about 6 cm. water, with very small respiratory fluctuations, while the venous pressure near the entry of the duct was 2·4 cm. water. When a cannula filled with water was tied into the cephalic end of the divided thoracic duct, water flowed into the vein during inspiration only. They concluded that in the intact animal lymph was aspirated into the veins during inspiration. On the other hand, when the main thoracic duct *is cannulated and all collateral vessels tied off, lymph flows,* against atmospheric pressure, into the cannula more rapidly during expiration, with the animal supine, and may not flow at all during inspiration when the animal is tilted head upwards.

A factor of considerable importance, however, in the transfer of lymph along the thoracic duct through the thoracic cavity is the actual pressure on the cisterna chyli. Pressure on the abdominal wall over the cisterna chyli will, for a time, greatly accelerate the flow from a cannula in the duct Any considerable increase in abdominal pressure, such as coughing, straining or lifting, will therefore have a powerful effect on propelling the lymph along the thoracic duct from the abdominal cavity through *the thoracic cavity to the base of the neck.* This vis-a-tergo transmitted from more distal segments is no doubt important in lymph transfer along other channels too. The lymphatics are easily distended, yet the valves will often remain competent in the face of high pressures. This enables pressure to be stored in distended segments and released

in cervical lymph flow following electrical stimulation of the chorda tympani, although a copious flow of saliva was obtained.

The intestinal lymph flow as a rule rises during activity of the digestive tract. This is due in part to the absorption of fluid. What rôle the activity of the glandular tissue plays in the increased lymph flow is not clear. It is well known that the digestion and absorption of fat increases the flow, while a meal containing protein and carbohydrate but practically no fat, has no appreciable effect on lymph flow (Simmonds, 1955). The exact mechanism concerned in the increased lymph flow during fat digestion has not yet been elucidated.

Local temperature. The blood flow through the skin varies greatly with temperature. When a limb is placed in a water bath at 40 to 45° C. the blood flow and capillary filtration increase. The flow of lymph at the same time becomes greater. On lowering the temperature again, the blood flow decreases and the lymph flow is reduced. Within physiological limits, therefore, the lymph flow from the skin will vary with the degree of vasodilatation and blood flow (Courtice, 1946). In the same way, the lymph flow from the nasal mucosa may be increased by irrigation with saline at 45° C. The flow falls again when the temperature is decreased (McCarrell, 1939–1940) Temperatures higher than 50° C. lead to irreversible changes due to damage to the capillary membrane (see Chapter 8)

Other causes of alterations in the rate of formation of tissue fluid, such as venous obstruction, low plasma protein level, anoxia, and tissue injury have been discussed in some measure in Chapter 2 or will be discussed in Chapter 8.

Lymphagogues. In 1891, Heidenhain cannulated the thoracic duct in dogs, and found great increases in the solids of the lymph and in lymph flow following the intravenous injection of such complex substances as extracts of crayfish muscles, of leech heads, of the bodies of mussels, of the intestine and liver of dogs as well as such substances as peptone and egg white. He believed that these substances were capable of causing a secretion of lymph by a specific stimulating effect on the lymphatic endothelium, and he called these substances "lymphagogues". Our present knowledge of lymph formation, however, indicates that the endothelial cells have no such secretory function. Substances injected into the blood stream may increase lymph flow by increasing the rate of capillary filtration This is brought about either by damaging the capillary membrane, by raising the capillary blood flow and pressure without any alteration in permeability, or by both. Most of the substances which Heidenhain called "first class" lymphagogues were foreign proteins or peptones which increased lymph flow by damaging the capillary membrane. Drinker and Field (1933) have tabulated these

Fulton (1938) observed increased thoracic duct lymph flow in anaesthetized dogs given intravenous injections of pituitrin and pilocarpine. Muscarin produces the same result.

Beznak (1937) found that intravenous injection of acetylcholine caused a very prompt increase in thoracic duct lymph flow in cats and dogs. Observing the villi directly under the microscope during the action of the drug, he noted marked dilatation of the precapillary arteries and small but distinct dilatation of the capillaries—a reaction calculated to increase filtration and so increase the amount of tissue fluid. Though acetylcholine caused contraction of the villi, this was not responsible for lymph flow increase, since the drug still augmented lymph flow when the villi were paralysed by chlorazol skyblue.

Increased tissue fluid formation and lymph flow

Tissue activity. Other things being equal, the rate of capillary filtration in any tissue will be increased by an increase in the filtering head of pressure, by an increase of blood flow through the tissue or by an increased permeability of the capillary membrane. Under normal circumstances the blood flow through a tissue may depend to a large extent upon its activity. Drinker *et al.* (1940) showed that the lymph flow from the heart rose when its activity was increased by adrenaline. In muscular exercise we know that the lymph flow from a cannula, inserted in the lymph duct just above the ankle or wrist, is increased (White, Field and Drinker, 1933), likewise the lymph flow from the thoracic duct is increased (Cain *et al*, 1947) It is impossible to say, however, how much of this increase actually comes from the muscles.

We have very little reliable evidence to show whether lymph flow from a glandular tissue is increased during activity. Asher (1899) cannulated the thoracic duct in dogs, and reported an increase in lymph flow as a result of intravenous injections of bile and other substances, which are inferred to have increased the production of lymph by the liver. Bainbridge (1902) reported similar results following the injection of sodium taurocholate, and declared that while thoracic duct lymph increased after such intravenous injections, the result was negative if the periportal lymphatics were ligated. Falloise (1903) and Bainbridge (1905) cannulated the thoracic duct in dogs and injected secretin intravenously. They found an increase in lymph flow coinciding with secretion of pancreatic juice; and since this persisted after ligation of portal lymphatics, they concluded that the increased lymph came from the pancreas. There is no reason, however, why the increased lymph may not have come from the intestines. Leigh (1935) has reviewed the conflicting literature concerning lymph flow from the salivary glands during salivary secretion. He also reported experiments in which he found no increase

increases the lymph flow from the leg (Haynes, 1932*b*), and when injected intravenously acetyl-choline may cause a rise in thoracic duct lymph flow (Beznak, 1937). Noradrenaline has a complex action on lymph flow in various regions of the body, by its action in redistributing the blood from the high-pressure to the low-pressure systems (Korner, 1953; Korner and Courtice, 1954; see also Chapter 2)

It is clear, therefore, that the term "lymphagogue" should no longer be used in its original sense Since the many substances, which increase lymph flow when injected into the blood stream, do so in many different ways and not in any specific manner, as Heidenhain thought, it would be better not to use the word at all.

Age. The total water content of the several tissues of the body is greater in young than in adult animals (see Chapter 2) One of the suggestions offered to explain the scant flow of lymph from some dogs as contrasted with the copious flow from others is this factor of age, since it is reasonable to expect more lymph when there is a large supply of tissue fluid. Emminghaus in 1873 cannulated ankle lymphatics in dogs, and reported that he was unable to get reasonable quantities of lymph except from young animals; Holman (1937) collected lymph from the wrist and ankle lymphatics of dogs under local anaesthesia and found that the lymph flow was about twice as great in growing as in mature animals. Once maturity was reached, however, there was no significant change, even in very old dogs. As we mentioned in Chapter 1, a small young animal will generally produce more lymph than a much larger, old one.

Effect of anaesthesia on lymph flow

Most animal experiments are complicated in some way by the action of the anaesthetic. Lymph flow may be affected by anaesthesia because of the lack of muscle tone and muscle contraction, and a change in rate or depth of respiration. That is, the factors responsible for the propulsion of lymph along the lymphatic channels have been altered Anaesthesia may also alter the filtration through the capillary membrane of a region either by changing the capillary pressure or the capillary permeability. Changes in lymph flow will run parallel with degree of capillary filtration. Polderman, McCarrell and Beecher (1943) investigated the effect of ether and of barbiturate anaesthesia on the lymph flow from the cervical ducts of dogs The flow was produced by uniform passive motion of the head by means of a head-rocker. Compared with local anaesthesia only (procaine HCl), ether increased the flow while pentobarbital sodium reduced it (Table 31) The changes in blood and lymph in Table 31 suggest that under barbiturates fluid moves from the tissues into the blood stream, with the reverse process occurring under

substances. Included in this table is histamine, which dilates the capillaries and increases their permeability with a resultant increase in lymph flow, Fig. 74 (Dale and Laidlaw, 1911–1912; Beck, 1924; Haynes, 1932*b*; Paton, 1954). The importance of histamine lies in the fact that in tissue injury it is liberated from the damaged cells and plays a prominent rôle in the subsequent local hyperaemia and oedema formation (Lewis,

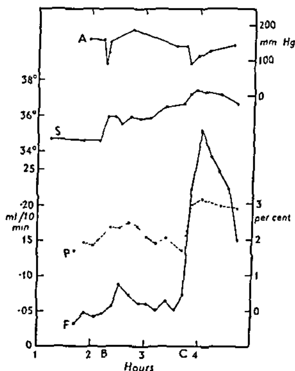


FIG 74 —The effect of acetylcholine and histamine on the arterial blood pressure, skin temperature, lymph flow and protein content of leg lymph in the dog

A Carotid B P mm Hg

At *B*, 8 ml
At *C*, 8 ml

(From Haynes, 1932*b*, *Amer J Physiol*)

1927; Dale, 1929; Rosenthal and Minard, 1939; Kellaway and Rawlinson, 1944, Rawlinson and Kellaway, 1944).

Apart from this group of substances, there are others which alter the haemodynamics without affecting appreciably the permeability of the capillaries. We have seen that intravenous transfusions of solutions of sodium chloride, glucose or even of plasma lead to increased filtration and lymph flow (see Chapter 2). Various drugs may also act in this way. For example, acetyl choline, a vasodilator, injected into a leg artery

widely as the muscles contract and relax. During muscle contraction the lymphatics empty while during relaxation they fill again. We must realize, therefore, that lymph pressure in any vessel, like lymph flow, is very variable throughout the course of a day. Measurements of lymph pressure have been made, however, in standard conditions of rest, with the animal under anaesthesia.

Thoracic duct pressure

Many of the measurements of lymph pressure in the thoracic duct are end pressure determinations. The pressures so recorded are interesting as showing what may happen if the duct is obstructed; but they do not measure the normal pressure of the fluid as it flows through the duct. Lee (1923–1924) found an average end pressure in the cannulated duct of dogs of 15 cm. water (11 mm Hg). The pressure is greatly influenced by pressure upon the abdomen over the cisterna chyli or by a sudden bout of coughing. Under forced breathing, it reached 35 cm water (26 mm. Hg)

Rouvière and Valette (1937) measured the side pressure by cannulating a division of the thoracic duct, often found in the dog at the point of entrance into the subclavian vein, and obtained a pressure of 6·4 cm. water. Pressure in the internal jugular vein of the same animal was 2·4 cm. water The difference between lymph pressure and venous pressure promotes ready entrance of lymph into the veins at the base of the neck. Webb and Starzl (1953) found side pressures of 3·5 to 5·5 cm. water in the thoracic duct just above the diaphragm in anaesthetized dogs. At this point, arterial pulsations affected the lymph pressure, the difference between the pressures during systole and diastole being 2 to 3 cm. water.

Cervical lymphatic pressure

McCarrell (1939*b*) inserted a T-cannula in a cervical lymphatic in dogs and measured lateral pressures during passive motion of the head. Pressures ranging from − 2·8 to 3·2 cm. water were obtained, with the majority of readings falling between 0·1 and 1·4 cm Venous congestion produced by clamping the external jugular veins, though causing increased flow, affected the pressure but slightly. End pressures, obtained by end cannulation of cervical lymphatics, reached values as high as 44·5 cm water. Rouvière and Valette (1937) used an indirect method—the measurement of pressure required to collapse cervical lymphatics—and obtained pressures of 1·5 to 2 cm. water. Figures of similar range were reported by Noll as long ago as 1850 and by Weiss in 1861. Webb and Starzl (1953) obtained side pressures of 2·5 to 10 cm. water in anaesthetized dogs.

TABLE 31

The effect of pentobarbital sodium and of ether on the lymph flow from the [illegible] aesthesia only. Flow [illegible] In the upper table, [illegible] he lower table ether anaesthesia was followed by barbiturate.

	Procaine HCl	*Pentobarbital Na*	*Ether*
Lymph flow mg /min.	117·4	63 6	186 3
Lymph protein g %	2 46	2 68	2 21
Haematocrit	40 6	37 0	46·3
Plasma protein g %	6 01	5 70	6 26
Blood pressure mm Hg	132	132	138
Respiration rate	28	17	24
	Procaine HCl	*Ether*	*Pentobarbital Na*
Lymph flow mg /min	111 8	169 4	42·3
Lymph protein g %	2 87	2 64	3 26
Haematocrit	41·4	50 0	47 8
Plasma protein g %	6·46	7 17	6 57
Blood pressure mm Hg	122	127	113
Respiration rate	28	66	66

From Polderman, McCarrell and Beecher (1943)

ether. Hungerford and Reinhardt (1950) found that the thoracic duct lymph flow of 40-day old rats under ether anaesthesia was 30 per cent greater than under pentobarbital sodium, but this difference was not observed in 60-day old animals. In dogs, Cain *et al.* (1947) found that ether anaesthesia increased the lymph flow from the liver and from the thoracic duct, but in another series of dogs Nix *et al* (1951) obtained larger volumes of lymph after the animals had recovered from ether anaesthesia In general, therefore, it seems that barbiturates reduce the lymph flow and increase the concentration of lymph proteins whereas ether has the reverse effect. These changes are probably brought about by alterations in the tone of the blood vessels and therefore in the distribution of blood in the various tissues.

Thoracic duct lymph flow

The lymph flow from the thoracic duct, unlike that from other ducts of the body, is considerably affected by absorption from the small intestine. This has been fully discussed in Chapter 3.

LYMPH PRESSURES

It is clear that lymph flows centripetally along a duct from a region of higher pressure to a region of lower pressure and that muscle contraction may greatly increase the tissue pressure and the lymph pressure in any region. For example, in walking the pressures in the leg fluctuate

present only figures obtained in fairly recent times and by reliable methods Wherever possible, data on both lymph and blood plasma or serum from the same animal or the same groups of animals are given, since comparisons of lymph with blood plasma are so often necessary in considering the problems offered by lymph.

Proteins in lymph

In the discussion, in Chapter 2, of the formation of lymph, we have concluded from chemical and electrophoretic analyses that lymph contains all the protein fractions of plasma, but at a lower level. The concentrations of these fractions in lymph depend upon several factors which have already been fully considered. In Table 32 are assembled some of the recorded data for the total protein, albumin and globulin in lymph collected from various lymphatic ducts of the body. The various authors have used different methods for the estimation of protein and for the separation of albumin and globulin. Most of the values listed have been obtained with the animal under a barbiturate anaesthetic, although in some cases ether was used or lymph was obtained from the unanaesthetized animal. In general, however, the figures obtained are fairly constant for any region of the body. We see that relative to the plasma level the concentration of protein is highest in liver lymph and lowest in lymph from the skin.

A preparation that has been used to a great extent in recent years for the study of lymph is the rat with a thoracic duct fistula (Bollman, Cain and Grindlay, 1948). In this preparation lymph may be collected for several days. The concentration of protein in the lymph, however, varies considerably, depending on the composition and quantity of fluid the rat is allowed to drink. If a saline solution is made freely available, the rat will drink copiously and produce a very large amount of lymph with a low protein concentration (cf Shrewsbury and Reinhardt, 1952, and Table 18 in Chapter 3) The larger the volume of saline solution drunk, the greater the lymph flow and the lower the protein level. The value of giving saline especially in the early stages is to promote flow and prevent coagulation which would block the transflex tubing The figures obtained for the concentration of protein in the lymph from this preparation do not, therefore, necessarily represent the true values in the normal animal.

Coagulation properties. Lymph from all parts of the body clots, but as a rule less readily than does plasma. Howell (1914) studied the coagulation of thoracic duct lymph from the dog, and found varying coagulation times, the longest occurring when the lymph was heavily loaded with fat. The concentrations of fibrinogen and of prothrombin in lymph are always less than in plasma and vary considerably in different

Pressure in leg lymphatics

Drinker and Field (1933) placed a T-cannula in a lymphatic just below the popliteal lymph node in a dog. No pressure was recorded when the animal was at rest; but when the foot was attached to a rotator so that rapid flexion and extension occurred, the pressure rose to 68 cm water When the vessel was completely obstructed, pressures as high as 99 cm. of water were obtained.

Cardiac lymphatic pressure

It has not been possible to obtain a lateral pressure determination in the efferent cardiac lymphatic. End pressure has, however, been measured (Drinker *et al.*, 1940). With the dog's heart beating normally and with a mean blood pressure of 112 mm. Hg, lymph pressure became steady at 15 5 cm. water. After intravenous injection of adrenalin, the pressure became 18·6 cm.

Pressure in lymphatics of villi

Königes and Otto (1937), using cats anaesthetized with dial, made measurements by the direct technique of Landis (1925–1926), with modifications, of the pressure in the blood capillaries of the villi and of pressure in the central capillary lymphatic. They obtained interesting figures, rather higher than one would have expected. The pressure in the precapillary of the villus was 50·7 cm. water (37·3 mm Hg), in the actual capillary 42·6 cm. water (31·3 mm. Hg); and in the postcapillary 33 cm water (24·3 mm. Hg) The pressure in the lymphatic of the villus was 33·3 cm. water (24 5 mm Hg).

Chemical Composition of Lymph

In previous chapters we have shown that lymph forms part of the extracellular fluid or environmental fluid system of the body. The basic chemical structure of lymph, therefore, closely resembles that of plasma While discussing lymph formation in the various tissues of the body, we have repeatedly referred to the composition of the lymph. In this chapter, however, we propose to assemble in a few tables some of the reliable figures now available. Medical literature contains numerous analyses of lymph, some of which are now more than a hundred years old Such data are of historical interest, but have been obtained by methods long obsolete. Those interested in the early literature on the composition of lymph in man and in different animals should consult Nasse (1843), von Gorup-Besanez (1874), Hoppe-Seyler (1881) and Munk and Rosenstein (1891). In our tables we have endeavoured to

	,,	,,	5·67	2·79	3·47	1·90	1·62	0·64	Ether	Nix, Mann, Bollman, Grindlay and Flock (1951)
	Cat	(5)	6·60	5·26	3·11	2·62	3·49	2·64	Nembutal	Morris (unpublished data)
Right lymph duct	Dog	(21)	5·40	3·70	—	—	—	—	,,	Courtice (1951)
	Cat	(1)	7·40	4·90	—	—	—	—	,,	Courtice (unpublished data)
Lung lymphatics	Dog	(18)	—	3·66	—	—	—	—	,,	Warren and Drinker (1942)
Cardiac lymph	,,	,,	—	3·69	—	—	—	—	,,	Drinker, Warren, Maurer and McCarrell (1940)
	,,	(6)	5·94	3·83	2·98	2·20	2·96	1·63	,,	Drinker, Warren, Maurer and McCarrell (1940)
Cervical duct	Monkey	(4)	5·12	3·48	—	—	—	—	,,	Yoffey (unpublished data)
	Dog	(13)	6·25	3·63	3·61	2·36	2·63	1·26	,,	Field, Leigh, Heim and Drinker (1934–1935)
	,,	(16)	6·18	3·32	—	—	—	—	,,	Heim (1933)
	,,	(11)	6·26	2·68	—	—	—	—	Local	Polderman, McCarrell and Beecher (1943)
	,,	,,	6·76	2·44	—	—	—	—	Ether	Polderman, McCarrell and Beecher (1943)
	,,	,,	6·17	3·00	—	—	—	—	Nembutal	Polderman, McCarrell and Beecher (1943)
	,,	(3)	5·65	2·57	3·67	1·72	1·97	0·85	,,	Courtice and Morris (1955)
	,,	(11)	5·52	2·63	—	—	—	—	,,	Courtice (unpublished data)
	Cat	(2)	—	4·09	—	—	—	—	,,	McCarrell (1940)
	,,	(6)	7·09	3·71	3·65	2·44	3·44	1·05	,,	Morris (unpublished data)
	Rabbit	(8)	—	3·18	—	—	—	—	,,	Yoffey (unpublished data)
	Guinea-pig	(1)	4·64	3·37	—	—	—	—	,,	Drinker and Yoffey (unpublished data)
Renal lymph	Dog	(11)	5·81	1·84	—	—	—	—	,,	Sugarman, Friedman, Barrett and Addis (1942)
Leg lymphatics	,,	(8)	6·46	1·91	3·62	1·20	2·84	0·71	Nembutal	Field, Leigh, Heim and Drinker (1934–1935)
	,,	(11)	5·62	1·72	—	—	—	—	,,	Courtice (unpublished data)
	,,	(4)	7·38	1·41	—	—	—	—	Sodium barbital	Drinker and Field (1931)
	,,	(7)	—	1·52	—	—	—	—	Local	Weech, Goettsch and Reeves (1933)
	,,	(8)	—	0·5–1·52	—	—	—	—	,, (walking)	White *et al* (1933)
	Cat	(7)	—	3·31	—	—	—	—	Nembutal	Yoffey (unpublished data)
	Rabbit	(2)	—	1·26	—	—	—	—	,,	Drinker and Yoffey (unpublished data)

TABLE 32

The protein content of lymph from various regions of the body compared with that of plasma

Source of Lymph	Animal	Total Protein g/100 ml		Albumin g/100 ml		Globulin g/100 ml		Anaesthetic	Authors
		Plasma	Lymph	Plasma	Lymph	Plasma	Lymph		
Thoracic duct	Human (5)	7·08	4·89	2·86	2·34	4·16	2·56	Nil	Bierman, Byron, Kelly, Gilfillan, White, Freeman and Petrakis (1953)
	" (1)	6·00	2·80–3·60	3·50	1·64–2·45	2·50	1·16–1·15	"	Courtice, Simmonds and Steinbeck (1951)
	" "		3·19–5·28	—	—	—	—	"	Crandall, Barker and Graham (1943)
	Monkey (3)	5·87	3·66	—	—	—	—	Nembutal	Yoffey (unpublished data)
	Dog (11)	6·19	4·00	3·56	2·45	2·62	1·54	"	Field, Leigh, Heim and Drinker (1934–1935)
	" (6)	5·91	3·23	3·33	2·04	2·08	0·88	Ether	Nix, Mann, Bollman, Grindlay and Flock (1951)
	" "	6·11	3·67	—	—	—	—	Nil	Glenn, Cresson, Bauer, Goldstein, Hoffman and Healey (1949)
	" (3)	5·65	3·44	3·67	2·38	1·97	1·08	Nembutal	Courtice and Morris (1955)
	Cat (20)	7·09	4·63	3·65	2·74	3·44	1·88	"	" " " "
	Rabbit (15)	—	3·53	—	—	—	—	"	Drinker and Yoffey (unpublished data)
	" (10)	5·46	3·43	3·56	2·22	1·89	1·20	"	Courtice and Morris (1955)
	Rat (10)	5·82	3·06	3·87	1·90	1·95	1·16	Nil	Nix, Flock and Bollman (1951)
Liver lymph	" (5)	5·68	1·94	3·20	1·29	2·47	0·65	"	Morris (unpublished data)
	Dog (3)	6·34	5·32	3·38	2·89	2·96	2·51	Nembutal	Field, Leigh, Heim and Drinker (1934–1935)
	" (13)	5·67	4·39	3·41	2·74	1·81	1·28	Ether	Nix, Mann, Bollman Grindlay and Flock (1951)
	Cat (2)	5·28	5·17	3·38	3·15	1·90	2·02	Nembutal	McCarrell, Thayer and Drinker (1941)
Gall-bladder lymph	" (5)	6·60	6·12	3·11	2·92	3·49	3·20	"	Morris (unpublished data)
Intestinal lymph	" (2)	5·28	5·01	3·38	3·18	1·90	1·83	"	McCarrell, Thayer and Drinker (1941)
	Dog (2)	6·24	3·98	3·67	2·42	2·57	1·56	"	Field, Leigh, Heim and Drinker (1934–1935)
	" (10)	5·98	2·97	3·18	1·72	2·80	1·25	Sodium barbital	Wells (1932)

	"	"	5·67	2·79	3·47	1·90	1·62	0·64	Ether	Nix, Mann, Bollman, Grindlay and Flock (1951)
	Cat	(5)	6·60	5·26	3·11	2·62	3·49	2·64	Nembutal	Morris (unpublished data)
Right lymph duct .	Dog	(21)	5·40	3·70	—	—	—	—	"	Courtice (1951)
	Cat	(1)	7·40	4·90	—	—	—	—	"	Courtice (unpublished data)
Lung lymphatics .	Dog	(18)	—	3·66	—	—	—	—	"	Warren and Drinker (1942)
Cardiac lymph .	"	"	—	3·69	—	—	—	—	"	Drinker, Warren, Maurer and McCarrell (1940)
	"	(6)	5·94	3·83	2·98	2·20	2·96	1·63	"	Drinker, Warren, Maurer and McCarrell (1940)
Cervical duct .	Monkey	(4)	5·12	3·48	—	—	—	—	"	Yoffey (unpublished data)
	Dog	(13)	6·25	3·63	3·61	2·36	2·63	1·26	"	Field, Leigh, Heim and Drinker (1934–1935)
	"	(16)	6·18	3·32	—	—	—	—	"	Heim (1933)
	"	(11)	6·26	2·68	—	—	—	—	Local	Polderman, McCarrell and Beecher (1943)
	"	"	6·76	2·44	—	—	—	—	Ether	Polderman, McCarrell and Beecher (1943)
	"	"	6·17	3·00	—	—	—	—	Nembutal	Polderman, McCarrell and Beecher (1943)
	"	(3)	5·65	2·57	3·67	1·72	1·97	0·85	"	Courtice and Morris (1955)
	"	(11)	5·52	2·63	—	—	—	—	"	Courtice (unpublished data)
	Cat	(2)	—	4·09	—	—	—	—	"	McCarrell (1940)
	"	(6)	7·09	3·71	3·65	2·44	3·44	1·05	"	Morris (unpublished data)
	Rabbit	(8)	—	3·18	—	—	—	—	"	Yoffey (unpublished data)
	Guinea-pig	(1)	4·64	3·37	—	—	—	—	"	Drinker and Yoffey (unpublished data)
Renal lymph .	Dog	(11)	5·81	1·84	—	—	—	—	"	Sugarman, Friedman, Barrett and Addis (1942)
Leg lymphatics .	"	(8)	6·46	1·91	3·62	1·20	2·84	0·71	Nembutal	Field, Leigh, Heim and Drinker (1934–1935)
	"	(11)	5·62	1·72	—	—	—	—	"	Courtice (unpublished data)
	"	(4)	7·38	1·41	—	—	—	—	Sodium barbital	Drinker and Field (1931)
	"	(7)	—	1·52	—	—	—	—	Local	Weech, Goettsch and Reeves (1933)
	"	(8)	—	0·5–1·52	—	—	—	—	(walking)	White et al (1933)
	Cat	(7)	—	3·31	—	—	—	—	Nembutal	Yoffey (unpublished data)
	Rabbit	(2)	—	1·26	—	—	—	—	"	Drinker and Yoffey (unpublished data)

regions, just as the concentrations of other proteins vary. Brinkhous and Walker (1941) found that in eight dogs the mean prothrombin level, expressed as a percentage of that in the plasma, was 93·2 in the liver lymph, 51·2 in thoracic duct lymph and 7·6 in leg lymph. The mean fibrinogen level was 211 mg. per cent in the thoracic duct lymph compared with 410 mg. per cent in the plasma. Fantl and Nelson (1953) found similar figures in the thoracic duct lymph of the dog.

Of the other coagulation factors, calcium is slightly lower in the lymph than in the plasma, but platelets, which are regarded as supplying thromboplastin in blood coagulation, are absent. The lymphocytes, the principal cells of the fluid, are not conspicuous for thromboplastic activity. Fantl and Nelson found that thoracic duct lymph of dogs clots in far shorter time when in contact with glass than when in contact with a water-repellant siliconized surface. Since there are no platelets in the lymph, they explain the initial stages of the clotting process of lymph by the presence of thromboplastin precursors whose rate of conversion into active thromboplastin depends on the surface properties of the container.

Antibody content of lymph. Chemical fractionation of the plasma proteins by the methods of Cohn and his associates indicates that the antibodies are largely present in Fraction II and to some extent in Fraction III Electrophoretically, they migrate mainly as γ-globulins, but some also migrate with the faster moving β-globulins. We have observed in earlier chapters that all the fractions that can be distinguished by electrophoresis are present in the lymph as well as in the plasma. We should expect, therefore, that, as with the other plasma proteins, the antibodies would take part in the extravascular circulation (see p. 84), and that the concentrations of antibodies in the lymph relative to those in the plasma would correspond fairly well with the total protein content.

Hughes and Carlson (1908) found haemolysins for rabbit's red cells present in the following descending order: serum; thoracic duct lymph; cervical lymph; leg lymph; pericardial fluid; aqueous humour. This arrangement is in direct relation to protein concentration. Following the intravenous injection of antisera, Becht and Luckhardt (1916) found higher concentrations of antibodies in thoracic duct lymph than in cervical lymph. Freund and Whitney (1929) found that when rabbit serum containing agglutinins is injected into the ear vein of rabbits, agglutinins accumulate in the lymph of the liver very rapidly, and in the lymph of the leg very slowly.

Although the antibodies in the lymph come mainly from the blood stream and so form part of the extravascular circulation of proteins, they in part originate in lymph nodes (see Chapter 5) and reach the blood

stream by way of the lymphatic vessels. The lymph in the efferent vessels of a node may, therefore, under certain circumstances, contain a greater amount of antibody than the lymph in the afferent vessels of a lymph node (Ehrich and Harris, 1942). The efficacy of intravenously injected antibodies will be discussed in Chapter 8.

Enzymes in lymph. Many enzymes have been recognized in the blood plasma, but not in high concentrations. They comprise probably less than 0·1 per cent of the plasma proteins (Surgenor, Hunter and Brown, 1953). Like the antibodies, these enzymes have most probably been produced by cells and transported to the blood stream. Once in the blood stream they take part in the extravascular circulation of plasma proteins, so the lymph collected from various regions of the body has

TABLE 33

Enzymes in lymph

Enzyme	*Source of Lymph*	*Animal*	*Author*
Amylase	Chyle	Man	Munk and Rosenstein (1891)
Maltase	Thoracic duct	Dog	Bial (1892)
Amylase and lipase	Chyle	Man	Hamill (1906–1907)
Diastase	Thoracic duct, neck and leg lymphatics	Dog	Carlson and Luckhardt (1908–1909)
Diastase and maltase	Thoracic duct	,,	Osato (1920)
Protease and lipase	,, ,,	,,	,, (1921b)
Catalase	Leg lymphatics	Rabbit	Ishino (1933)
Lipase (Butyrase)	,, ,,	,,	,, (1934)
Peptidase	,, ,,	,,	,, (1935–1936a)
Saccharase	,, ,,	,,	,, (1935–1936b)
Cholinesterase	Thoracic duct and cervical lymphatics	Dog	Friend and Krayer (1951)
,,	Thoracic duct, liver, cervical and mesenteric lymphatics	,,	Brauer and Hardenbergh (1947)
Alkaline phosphatase	Thoracic duct	,,	Gonzalez-Oddone (1946)
,, ,,	Intestinal lymph	Rat	Flock and Bollman (1948; 1950b)
Histaminase	Thoracic duct	Cat	Carlsten and Wood (1951)
Amylase	Intestinal lymph	Rat	Flock and Bollman (1950a)
Tributyrinase	,, ,,	,,	,, ,, ,, (1950a)

been found to contain many enzymes, Table 33. The enzyme concentration in the lymph in general is therefore usually lower than that in the plasma and the concentration runs parallel with the concentration of protein. The cholinesterase activities of blood plasma and of lymph from the liver, thoracic, intestinal and cervical ducts of dogs were measured by Brauer and Hardenbergh (1947) while Friend and Krayer (1941) determined the cholinesterase activity of plasma and of thoracic and cervical duct lymph. The level of cholinesterase activity of the lymph from these different regions of the body was, in general, proportional to the protein content, suggesting that the lymph cholinesterase has mainly diffused from the blood stream. Carlson and Luckhardt (1908–1909)

studied the level of diastase in blood, lymph and other fluids of the dog. They found that the concentration of diastase normally decreased in the following order: Serum, thoracic duct lymph, cervical and leg lymph, pericardial fluid, cerebro-spinal fluid. Flock and Bollman (1950*b*) showed that in the rat the amylase content of intestinal lymph was always less than that of the plasma, and was not related to feeding. They concluded from this that amylase was not absorbed from the intestine into the intestinal lymph during the digestion of food, nor was it secreted in increased amounts into the lymphatic vessels of the pancreas when this gland was secreting enzymes into the intestine. The evidence therefore suggests that the amylase of lymph represents that which has diffused from the blood stream. On the other hand, the concentration of tributyrinase in the intestinal lymph was not increased during a fat-free meal, but was considerably increased after the feeding of a fat-containing meal The concentration of tributyrinase in the plasma was diminished when the intestinal lymph was drained away from the body. It appears, therefore, that the level of this enzyme in the plasma of the rat is largely dependent on the lymph flowing from the intestinal mucosa during fat absorption.

The alkaline phosphatase content of intestinal lymph of the rat is higher than that of the plasma and increased after a fat-free meal, but much more so after a fat-containing meal. When all the intestinal lymph was drained away from the body, the concentration of alkaline phosphatase in the plasma was greatly reduced (Flock and Bollman, 1948). This suggests that the serum alkaline phosphatase comes to a large extent from the intestine and is transported to the blood stream mainly by the lymphatics When the bile duct was ligated or the bile drained away in a biliary fistula, the increase of alkaline phosphatase of the intestinal lymph following the feeding of fat was abolished or greatly diminished (Flock and Bollman, 1950*a*). The presence of bile is, therefore, important in the release of alkaline phosphatase from the intestinal mucosal cells with its subsequent absorption into the lymphatics.

It is clear from these observations that some enzymes, such as alkaline phosphatase and tributyrinase, can be shown to enter the blood stream from their cells of origin by way of the lymphatics. In other instances the mode of entrance into the blood stream has not been determined, but it would seem likely that they travel by the lymphatic vessels. Once in the plasma, all these enzymes take part in the circulation through the tissue fluid and lymph just as other proteins do.

Non-protein constituents of lymph

Electrolytes. Although the recorded analyses of the cations and anions of lymph are very scanty, it is clear that the ionic pattern of lymph

TABLE 34

Average values for the concentrations of electrolytes in lymph and plasma.

Substance	Source of lymph	Animal	Plasma	Lymph	Anaesthetic	Author
Sodium mEq/l	Thoracic duct	Man (5)	127	127	Nil	Bierman *et al.* (1953)
	Cervical duct	Dog	163	157	Nembutal	Lowry and Maurer (quoted by Drinker and Yoffey, 1941)
Potassium mEq/l	Thoracic duct	Man (5)	5·0	4·7	Nil	Bierman *et al.* (1953)
	Right lymph duct	Dog (5)	—	5·4	Nembutal	Courtice (unpublished data)
Calcium mEq/l	Thoracic duct	Man (5)	5·0	4·2	Nil	Bierman *et al.* (1953)
	" "	Dog (1)	5·2	4·6	Amytal	Arnold and Mendel (1927)
	Cervical duct	" (11)	5·8	4·9	Nembutal	Heim (1933)
Chloride mEq/l	Thoracic duct	Man (5)	96	98	Nil	Bierman *et al.* (1953)
	" "	Dog (1)	110	116	Amytal	Arnold and Mendel (1927)
	Cervical duct	" (7)	116	122	Nembutal	Heim (1933)
Inorganic P mg %	Thoracic duct	Man (5)	4·5	4·4	Nil	Bierman *et al.* (1953)
	" "	Dog (1)	4·3	3·6	Amytal	Arnold and Mendel (1927)
	Cervical duct	" (3)	5·6	5·9	Nembutal	Heim (1933)
Carbon dioxide ml/100 ml	" "	" (7)	56·8	58·8	"	Heim and Leigh (1935)
Carbon dioxide tension mm Hg.	" "	" "	46·4	40·3	"	" " " "
pH	" "	" "	7·34	7·41	"	" " " "

TABLE 35

The total fatty acid, total cholesterol and phospholipid phosphorus in the plasma and lymph of groups of animals *in the* postabsorptive state.*

			Total fatty acid mEq/l		*Total Cholesterol mg %*		*Phospholipid P mg %*			
			Plasma	*Lymph*	*Plasma*	*Lymph*	*Plasma*	*Lymph*	*Anaesthetic*	*Authors*
Thoracic Duct	Cat	(20)	10·8	9·1	98·4	43·6	7·4	4·0	Nembutal	Courtice and Morris (1955)
	Dog	(3)	14·0	13·1	258	124	16·0	8·9	„	„ „ „ „
	Rabbit	(10)	11·4	13·8	46	43	4·2	4·3	„	„ „ „ „
	Rat	(5)	10·4	9·6	51	72	5·5	8·1	Nil	Morris (unpublished data)
Cervical Duct	Cat	(6)	10·8	5·2	98·4	35	7·4	3·9	Nembutal	Courtice and Morris (1955)
	Dog	(3)	14·0	5·3	258	67	16·0	4·1	„	„ „ „
	„	(11)			137	56	—	—	„	Marble, Field, Drinker and Smith (1934)
Hepatic Duct	Cat	(9)	7·06	7·01	131	110	8·1	7·1	„	Morris (unpublished data)

* Samples were collected 24 hours after food in the dog and cat and 48 hours in the rat; rabbits were fed on a low-fat diet of vegetable leaves for a week to reduce alimentary absorption of fat to a minimum.

TABLE 36

Non-electrolyte composition of lymph compared with that in plasma.

Substance	*Source of lymph*	*Animal*	*Plasma*	*Lymph*	*Anaesthetic*	*Authors*
Glucose mg %	Thoracic duct	Dog (1)	123	124	Amytal	Arnold and Mendel (1927)
	Cervical lymphatics	" (16)	123 0	132 2	Nembutal	Heim (1933)
	" "	" (9)	103 5	101 9	"	Kaplan, Friedman and Kruger (1943)
	Renal lymphatics	" "	103 5	92 7	"	" " " " "
	Leg lymphatics	" (10)	111	115	Local	Heim, Thomson and Bartter (1935)
	Intestinal lymphatics	Cat (1)	219	219	Nembutal	" " " " "
N P N. mg. %	Thoracic duct	Dog (1)	27 2	27 0	Amytal	Arnold and Mendel (1927)
	" "	" (10)	40 0	39 0	Nembutal	Field, Leigh, Heim and Drinker (1934–1935)
	Cervical lymphatics	" (8)	37 5	37 4	"	" " " " " "
	Leg lymphatics	" (1)	36 0	37 3	"	" " " " " "
	Cervical lymphatics	" (10)	32 6	34 8	"	Heim (1933)
	Right lymph duct	" (20)	30 0	31 0	"	Courtice (unpublished data)
	Thoracic duct	" (4)	33 0	33 0	"	" " "
	" "	Cat (3)	45 0	44 0	"	Simmonds " "
Urea mg. %	Cervical lymphatics	Dog (7)	21 7	23 5	"	Heim (1933)
	Renal lymphatics	" (11)	53 1	69 7	"	Sugarman, Friedman, Barrett and Addis (1942)
Amino-acids mg. %	Cervical lymphatics	" (1)	4 90	4 84	"	Heim (1933)
	Thoracic duct	"		2 4	Local	Petersen and Hughes (1925)
Creatinine mg. %	Cervical lymphatics	" (7)	1 37	1 40	Nembutal	Heim (1933)
Total P mg. %	" "	" (6)	22 0	11 8	"	" "
Non-fermentable reducing substances mg %	" "	" (10)	5 5	5 8	"	Heim, Thomson and Bartter (1935)

is not essentially different from that of plasma Table 34 gives average figures for the concentrations of some of the electrolytes in plasma and in lymph collected mainly from the thoracic or cervical ducts. In general, the total base is slightly lower in the lymph than in the plasma while the chloride and bicarbonate levels are higher in lymph than plasma The direction of the differences in concentrations of these constituents is consistent with the existence of the Gibbs-Donnan equilibrium operating on two phases whose concentrations of non-diffusible ions (protein ions) differ.

Non-electrolytes

(*a*) *Lipids.* The lipids in the plasma and lymph have already been discussed in Chapters 2 and 3. Cholesterol and phospholipid, being mainly associated with protein as lipoprotein, are present in the lymph in concentrations which vary with the level of protein in the lymph (Courtice and Morris, 1955) The amount of neutral fat in the form of chylomicrons in the thoracic duct lymph depends mainly on the degree of fat absorption from the alimentary tract Table 35 gives figures for the lipid content of lymph in animals in the postabsorptive state, when fat absorption is reduced to a minimum. During fat absorption after a meal rich in fat, the levels in lymph from the thoracic duct rise considerably (Chapter 3).

(*b*) *Non-lipids* The readily diffusible non-protein constituents of the plasma are present in the lymph in concentrations which are approximately the same as in the plasma In Table 36 are given some of the values recorded in the literature. In a steady state the lymph glucose is, in general, the same as the plasma glucose, except in the kidney where the lymph has a slightly lower content After a carbohydrate meal when the blood sugar rises rapidly, or after the intravenous injection of glucose, the lymph sugar will lag behind; but, ultimately, equilibrium will again be reached when the levels are once more approximately the same (Robertson and Williams, 1939) The non-protein nitrogen, urea, amino-acid and creatinine levels are likewise approximately the same in both plasma and lymph except in the kidney where the lymph urea is slightly higher than in the plasma. The significance of the glucose and urea levels of renal lymph have already been discussed (p. 203).

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CHAPTER 5

BIOLOGICAL SIGNIFICANCE OF LYMPHOID TISSUE

By lymphoid tissue we understand any tissue consisting predominantly of lymphocytes, such as lymph nodes, tonsils, appendix, and intestinal nodules. As we have already noted, however, the lymphocytes in lymphoid tissue are usually intermingled with other cell elements, to which they may be either functionally or genetically related. A brief survey of the distribution of lymphoid tissue in vertebrates has already been given in Chapter 1, where it was stated that the segregation of the lymphoid from the myeloid tissues reaches its most advanced development in mammals, with the widespread formation, in intimate association with lymphatic vessels, of the typical mammalian lymph node. The formation of multiple lymph nodes serves to accentuate what was already a peculiar feature of the lymphoid tissues—namely, their scattering all over the body in a number of discrete masses.

Investigators seeking to determine the biological significance of these discrete masses have utilized a variety of methods of study. Of these we shall consider—from the point of view primarily of their effect on lymphoid tissue—ionizing radiation, toxic compounds, haemorrhage, growth in sterile surroundings, complete lymphatic blockage and varying degrees of surgical extirpation. The action of hormones on lymphoid tissue is discussed in Chapter 7.

FUNCTION OF LYMPHOID TISSUE: METHODS OF INVESTIGATION

Ionizing radiation

The diffuseness of distribution of the lymphoid tissues renders complete surgical removal impossible, though as we shall see (p. 264) experiments have been performed in which a very substantial portion of the total lymphoid mass has in fact been excised. Because of the difficulties of direct surgical approach, many workers have resorted to indirect methods of extirpation, such as exposure to X-rays, to which these tissues are particularly susceptible. For many years only external irradiation was available, mainly in the form of X-rays, as used in the pioneer work of Heineke (1904, 1905). More recently advances in nuclear physics have given investigators a much wider choice. Thus Bloom (1948) presents the work of a group of investigators who employed external irradiation by means of X-rays, β-rays emitted from P^{32}, γ-rays of radium and an

atomic pile, and fast and slow neutrons. Internal irradiation was effected by the administration of radioactive isotopes emitting α-, β- and γ-rays.

With all these indirect methods, however, it is not possible to be certain either that all the lymphoid tissues have been destroyed, or that other tissues have not been involved at the same time. In the case of X-rays, Hughes and Job (1937) specifically investigated this latter point, and found it impossible to administer a dose of X-rays sufficient to produce complete involution of all the lymphoid tissues, without at the same time damaging other tissues or organs. The results of this line of attack have not therefore been decisive; but they have nevertheless yielded results of considerable interest. Before discussing these briefly, it should perhaps be emphasized that it is not always easy to compare the results of different workers, partly because of species differences in sensitivity, partly by reason of variations in dosage and mode of administration (cf. Tullis and Warren, 1947, De Bruyn, 1948).

Lymphocyte sensitivity to radiation

In the early part of the century Heineke (1904, 1905) first described in detail the effects of irradiation on the haemopoietic system (mice, rats, guinea-pigs, rabbits, dogs). The most marked result which this author observed was degeneration of the lymphoid tissues, the earliest cells involved being lymphocytes and non-granular myelocytes (cf. Osgood *et al.*, 1942), subsequently he noticed also changes in eosinophils, mast cells and giant cells. Sturges and Levin (1920–1921) working with irradiated frogs demonstrated many years later that the sensitivity of lymphocytes to X-rays is equally marked in lower vertebrates and is, therefore, to be regarded as one of the fundamental biological properties of the lymphocytes. Lacassagne and Gricouroff, in 1927, showed that the action of X-rays on lymphocytes could be demonstrated *in vitro*, a finding subsequently confirmed by other workers (e g. Osgood, 1942, Trowell, 1952), though not in accord with the experiments of Jolly and Lacassagne (1923), who applied X-rays to blood *in vitro*, and found the vitality of the lymphocytes was not affected Cramer, Drew and Mottram (1921*a* and *b*) drew attention to the great similarity in the effects on lymphoid tissue of irradiation and vitamin B deprivation Mottram (1921–1922) found that not all lymphocytes responded to irradiation in the same way, but that the small lymphocytes were much more sensitive than the large.

Indirect action of X-rays

Jolly (1924*a* and *c*) noted that the sensitivity to X-rays of a lymph node or of the thymus could largely be abolished if the blood supply were

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adrenals, and the increased production of 11-oxysteroids, was suggested by Dougherty and White (1946), though it should be noted that previous workers (e g. Leblond and Segal, 1942; Gregoire, 1942) had already claimed that adrenalectomy exerted a beneficial effect upon the thymolymphatic atrophy produced by an intense localised irradiation. From a practical as well as a theoretical point of view, however, still greater interest has centred around the question of a humoral factor or factors which might have a directly beneficial effect upon recovery processes.

Jacobson *et al.* (1951) suggested that the spleen might play an important part, for they found that, in irradiated mice, shielding of the spleen practically doubled the animal's resistance to X-rays As long as the shielded spleen was present during the period of irradiation, then even though it was removed almost immediately afterwards a beneficial effect could still be noted. Furthermore, "Transplantation of one to four fresh spleens from young mice into the peritoneal cavity of mice immediately after exposure to 1,025r whole body X-radiation significantly increases the survival of the irradiated mice"

In many of these experiments it is possible that the spleen acts not by secreting a humoral factor, but by constituting a reservoir of undamaged cells which can travel via the blood stream to the various haemopoietic tissues. Rekers (1948) had been thinking along these lines when he transplanted healthy marrow cells into irradiated dogs, and the idea was subsequently followed up by Lorenz and his co-workers (e g Lorenz *et al.*, 1951) Congdon, Uphoff and Lorenz (1952) review the problem, and point out that the evidence is still not adequate to enable one to decide unequivocally whether the beneficial effect of administering bone marrow is due to a humoral factor, or is the result of repopulation with healthy cells.

Adhesion of lymphocytes to capillary walls

Another indirect effect of X-rays was described by Mottram (1931), who found that in irradiated rats there was a marked tendency for lymphocytes to adhere to the walls of the capillaries, a fact to which might be attributable their diminution in the circulating blood However, Fulton *et al.* (1954) directly examined the blood capillaries in the transilluminated cheek pouch of the hamster after total body irradiation, and concluded that "the leukopenia which persists for the first few days after irradiation is not due to leukocytes becoming immobile by sticking to the endothelium, or to their extravasation".

Differential response of lymph node and bone marrow

Jolly (1924*b*), studying the comparative sensitivity to X-rays of the popliteal lymph node and the femoral marrow (rabbit), found that the lymph node was very much the more sensitive of the two. Destructive

cut off by ligature. This suggests the possibility that X-rays may act indirectly by stimulating in some way the formation of toxic substances, which then circulate in the blood stream and so reach the node, an explanation which Jolly (1924*a*) considers unlikely. However, Leblond and Segal (1942), who also agreed that X-rays exerted an indirect effect, thought it was due to the development of a general intoxication following irradiation.

The extent and nature of the alleged indirect effect of X-rays upon lymphoid tissue has attracted a number of investigators. Osgood (1942) approached the problem through the medium of tissue culture of human marrow. "Marrow cultures were made in 4 vials, of which 2 were irradiated and 2 were not After irradiation the cultures were centrifuged and then the supernatant fluid from one of the irradiated and one of the non-irradiated cultures were withdrawn and interchanged." These experiments afforded no evidence of indirect action attributable to the liberation of toxic substances by the irradiated cells themselves. Osgood further suggests that where indirect action has been described, it may in fact be due to the transport of damaged cells, via the blood stream, from the irradiated tissue to that in which the indirect action is supposed to be exerted.

Parabiotic animals present another obvious line of experimental attack Barnes and Furth (1943), using both single and parabiotic mice, thought they could detect some evidence of indirect damage, but found it to be slight when compared with the changes produced by direct action. Lawrence, Valentine and Dowdy (1948) established a cross circulation in 7 pairs of cats, and then proceeded to irradiate one animal of each pair while the other was shielded Their results were "not considered to support the thesis of indirect effects peculiar to radiation". De Bruyn (1948*a*) also was unable to substantiate the indirect effect of X-rays. In a series of rabbits the right hind leg was irradiated, while the rest of the body was shielded with lead plates. "At 6 hours the popliteal node on the exposed side contained great masses of cellular debris. . . . Neither the popliteal lymph node on the shielded side nor the mesenteric lymph node showed any changes beyond those seen in the controls" Price (1948, 1951), on the basis of both human and animal studies, inclined to the view that there probably is an indirect effect of irradiation, and interprets the lymphopenia following irradiation in accordance with this concept.

Humoral factors in damage by or recovery from irradiation

For those workers who accept the hypothesis of the indirect action of X-rays, there arises the obvious question of a humoral factor or factors. A non-specific stress response, with stimulation of the pituitary and

acute and chronic application of externally originating ionizing radiations and internally deposited radio-isotopes have failed to reveal evidence in the blood-forming tissues and peripheral blood of a primary stimulation of haematopoiesis. However, secondary or 'compensatory increases' in certain of the cellular constituents of the peripheral blood were seen and were invariably preceded by a reduction." Warren, MacMillan and Dixon (1950) also observed, in mice which received minimal doses of P^{32}, a preliminary depression followed by hyperplasia of lymphoid and myeloid tissue, "with resultant increase in the size of the spleen, thymus, and amount of bone marrow but not in size of the lymph nodes which appear permanently damaged and shrunken by this small dose. This causes the lymphoid tissue of the nodes to present a peculiar contrast to its morphologic cousin, thymic tissue, which appears to have a much greater ability to come back after suffering equally severe irradiation damage."

Differential sensitivity of lymphocytes and other cells

The lymphoid tissues are the first to be affected by ionizing radiations. However, it is primarily the lymphocytes which are so sensitive, not the other elements, for as emphasized by De Bruyn (1948), there is a marked difference in sensitivity between lymphocytes on the one hand, and the reticular cells and macrophages on the other In rabbits 24 hours after 800r the medium-sized and large lymphocytes frequently showed signs of cellular and nuclear damage (clumping of chromatin, lobation of nuclei, formation of giant cells, etc.), while reticular cells and macrophages were not visibly affected Grégoire (1942), working with rats, has shown very convincingly in the case of the thymus that the reticular cells persist undamaged after the lymphocytes have been destroyed Furthermore, except with the smallest applications of X-rays, De Bruyn (1948) was unable to confirm the statement of Heineke (1904) that there is a latent period of 2–3 hours after exposure to X-rays before the destructive changes become evident. De Bruyn found that even 30–40 min after irradiation there was more nuclear debris in the nodes of the experimental rabbits than in those of the controls. However, with smaller doses of X-rays there was a latent period. Trowell (1952) notes that, like macrophages, monocytes and plasma cells also are much less sensitive than lymphocytes. Barrow, Tullis and Chambers (1951) showed that in rabbits, after whole body irradiation up to LD 50/30, there was no interference whatever with the uptake of colloidal gold by the reticulo-endothelial cells. Latta and Waggener (1954) found in rats given repeated injections of P^{32} that "The radioresistant reticular cells became more and more prominent in all hemopoietic tissues following injection as progressive hyperplasia recurred".

Martland, Conlon and Knef (1925), investigating the deposition of

changes were evident in the node immediately after irradiation, becoming intense in 2 to 3 hours. The marrow did not show marked changes until 12 hours after irradiation. Jolly (1925) also showed that marked histological changes could be produced in lymph nodes by direct exposure to ultra-violet rays

Taylor, Witherbee and Murphy (1919), and Thomas, Taylor and Witherbee (1919), measuring the effects of X-rays in terms of the circulating lymphocytes in a variety of animals, concluded that large doses produced a diminution in the circulating lymphocytes, whereas small doses caused them to increase, though after a varying interval of time. Mottram and Russ (1921) found, however, that there was no essential difference in this respect between small and large doses of X-rays, for in either case there was always an initial lymphopenia. After small doses, this lymphopenia lasted for a few hours, to be followed by a lymphocytosis; after large doses it persisted for a week or ten days Murphy (1926), discussing further the stimulant effect of one small dose of X-rays states that after the brief initial depression of the lymphocyte count, the blood lymphocytes almost doubled in number by the second day, and, as a rule, continued to increase until the fourteenth day. At the same time, an increased number of mitotic figures could be found in sections of lymphoid tissue from the fourth day onwards, with a return to normal about the tenth day. Latta and Ehlers (1931) review the literature and report additional experiments of their own. After several short exposures to X-rays (rats), totalling one hour, the blood leucocytes were reduced to one-fifth or less of their *normal value, the fall being most marked for the lymphocytes,* though affecting the neutrophiles also "Changes in the erythrocyte count appeared neither so rapidly nor in such degree as those noted in the leukocytes, although a very severe anaemia was produced eventually." The lymphoid tissue showed marked degenerative changes, and after longer irradiation, the lymphatic tissue of the spleen had practically disappeared, while the lymph nodes also showed extreme reduction in lymphocytes, the cortical regions consisting mainly of loose connective tissue. Goodfellow (1936) reviewed the clinical literature, and gave an account of his own observations on blood changes in patients subjected to irradiation. The consensus of opinion seemed to be that the haemopoietic organs in general are damaged by X-rays, but that the lymphoid tissues in particular were the most sensitive (see also Shouse, Warren and Whipple, 1931; Dunlap, 1942), and that the earliest and most marked destruction was observed in them.

Compensatory hyperplasia after irradiation

The question of cell proliferation after irradiation was carefully studied by Bloom and Jacobson (1948) who found that "Extensive studies with

acute and chronic application of externally originating ionizing radiations and internally deposited radio-isotopes have failed to reveal evidence in the blood-forming tissues and peripheral blood of a primary stimulation of haematopoiesis. However, secondary or 'compensatory increases' in certain of the cellular constituents of the peripheral blood were seen and were invariably preceded by a reduction." Warren, MacMillan and Dixon (1950) also observed, in mice which received minimal doses of P^{32}, a preliminary depression followed by hyperplasia of lymphoid and myeloid tissue, "with resultant increase in the size of the spleen, thymus, and amount of bone marrow but not in size of the lymph nodes which appear permanently damaged and shrunken by this small dose This causes the lymphoid tissue of the nodes to present a peculiar contrast to its morphologic cousin, thymic tissue, which appears to have a much greater ability to come back after suffering equally severe irradiation damage."

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radium and mesothorium in the tissues of workers with luminous (radioactive) paint, found that radium and mesothorium were taken up by reticulo-endothelial cells. Lymphoid tissue adjoining these cells—for example, *in the spleen—almost completely disappeared*: "... the spleen showed atrophic malpighian follicles, some of the terminal arterioles having hardly any surrounding lymphatic mantle." It would appear that the reticulo-endothelial cells with their radioactive contents form multiple small foci from which neighbouring lymphoid tissue is continuously irradiated.

Are all lymphocytes equally sensitive? Trowell (1952) reports that the small lymphocytes of lymphoid tissue are the most radio-sensitive cells in the body and that medium lymphocytes in this situation are almost equally sensitive. Trowell also finds that "Blood lymphocytes are less sensitive than lymph node lymphocytes, but not so insensitive as others have claimed". The scattered lymphocytes in the intestinal villi were found to be the least sensitive of all the lymphocytes examined.

On the other hand, as far as the lymphocytes in the various organized lymphoid masses are concerned, the response to irradiation appears to be identical in spleen, lymph nodes, or intestinal nodules of mice (Barrow and Tullis, 1952), and in the spleen, lymph nodes, tonsil, thymus, and intestinal nodules of swine (Tullis, 1951)

Barrow and Tullis (1952) concluded "that there is little difference in the degree to which lymphoid tissue is sensitive to a single exposure of total-body irradiation, whether the tissue be in the spleen, the lymph nodes, or the intestinal lymphoid aggregations". They also point out that variation in the degree of response of lymphoid nodules even in the same organ may be due to their being at different stages of the lymphopoietic cycle (Conway, 1937) In swine, Tullis (1951) observes: "The extent of the injury in the thymus was marked, but the proportion of fragmented lymphocytes to morphologically intact lymphocytes was much less in the thymus than in the other lymphoid organs." This observation is of especial interest in view of the findings of Warren, MacMillan and Dixon (1950) of more rapid regeneration of thymus than lymph nodes.

Time Sequence and Nature of Lymphoid Tissue Changes

Lawrence, Dowdy and Valentine (1848), after giving rats a single dose of 550r (whole body), found degenerative changes already present in lymphoid tissue within 1 hour after irradiation, while after 6 hours cytolysis in the lymphoid follicles had attained its maximum. By the end of 24 hours regeneration and repair of the lymph nodes was actively progressing, and after 20 days the regeneration of the lymph nodes was stated to be complete. However, even after 25 days the blood lymphocytes were still below their normal level.

De Bruyn (1948*b*) working with rabbits, rats and guinea-pigs, made a careful study of both degenerative and regenerative changes in the lymphoid nodules of lymph nodes and intestinal lymphatic tissue after varying dosage of X-rays. With regard to differences in dosage, he found that: "In rabbits doses of 800r and 600r completely destroy the [illegible]

regenerate rapidly and at 5 days are normal in appearance A dose of 100r does not produce a marked change in the majority of the nodules. Judging from the amount of debris, 50r is the lowest dose which produces histologically detectable changes."

In rats, De Bruyn found that doses of 600r and 400r produced changes identical with those observed in rabbits at these doses, whereas in guinea-pigs a dose of 175r produces changes resembling those found in rabbits and rats after doses in the neighbourhood of 400r. There appears to be no direct relationship between the damage to lymphoid tissue and the lethal action of X-rays upon the organism as a whole.

In rabbits after 800r regeneration first began after 5 days, when there re-appeared scattered collections of medium lymphocytes, many in mitosis The origin of these lymphocytes was obscure, and De Bruyn was not certain whether it was entirely homoplastic, or whether heteroplastic formation was also occurring.

Nodules began to re-appear after 21 days, first as small "bare" germinal centres, "consisting of medium-sized lymphocytes in mitosis, frequently associated with small areas of ectopic myelopoiesis". By 4 months typical nodules had re-formed, indistinguishable from those in the control animals.

Akaiwa and Takeshima (1950), investigating the effects of irradiation on the popliteal lymph node of the rabbit, came to the conclusion that after any but the smallest doses the lymph node never returned to normal, but finally underwent fibrosis Snell *et al* (1949), studying the haematological picture in a group of persons at Hiroshima 20–33 months after atomic bombing, found that the peripheral blood still showed several slight differences when compared with a control group, and that there was a "slight relative depression of lymphocytes and a slight elevation of eosinophiles".

Post-irradiation anaemia

The effects of irradiation on the bone marrow are of especial interest for their possible bearing on the problem of post-irradiation anaemia and recovery therefrom. It should be noted however that other factors may enter into the genesis of this anaemia than marrow damage alone. Ross,

radium and mesothorium in the tissues of workers with luminous (radio-active) paint, found that radium and mesothorium were taken up by reticulo-endothelial cells. Lymphoid tissue adjoining these cells—for example, in the spleen—almost completely disappeared: "... the spleen showed atrophic malpighian follicles, some of the terminal arterioles having hardly any surrounding lymphatic mantle." It would appear that the reticulo-endothelial cells with their radioactive contents form multiple small foci from which neighbouring lymphoid tissue is continuously irradiated.

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Furth and Bigelow (1952) studied the cell content of lymph in rats and dogs at varying periods after exposure to massive doses of X-rays. They found that both in lymph and blood there was a precipitous drop in lymphocytes within 5–10 hours after irradiation, but that in addition large numbers of erythrocytes appear in the lymph. This is merely an exaggeration of a normal process (see p. 324), but when it can lead to the presence of more than 1,000,000 erythrocytes in a c.mm. of lymph, it seems clear that this diversion of erythrocytes could be an important contributory factor in post-irradiation anaemia. It is tempting to explain the increased erythrocyte content of lymph as the result of damage to blood capillaries, with increased passage of red cells into lymphatics and their return to the blood via the main lymph ducts If this is the case, then experiments such as those of Ross *et al.* make it clear that such damage to the blood capillaries may have an unexpectedly delayed onset, since it was only after 7 days that the thoracic duct lymph began to contain greatly increased quantities of erythrocytes.

It has also been suggested that post-irradiation anaemia may be due to increased destruction of erythrocytes (Barrow and Tullis, 1952), or to the development of a haemorrhagic state associated with thrombocytopenia (Jackson *et al.*, 1952).

Changes in the Lymphocytes and their Significance

Schrek (1947) made a careful study of lymphocytes from the rabbit thymus suspended in homologous serum, incubated aerobically at 37° C. and then irradiated He described the formation of intranuclear vacuoles, which later discharged into the cytoplasm, leaving behind a dead pyknotic nucleus; and he concluded that the X-rays were accelerating a normal metabolic process, though without any evidence as to the nature of this process.

Stenstrom, King and Henschel (1942) investigated the effect of irradiation upon lymphocyte motility in tissue culture preparations of rabbit mesenteric lymph nodes. They found a group of small cells, no doubt lymphocytes, which were inhibited by doses up to 620r, and larger cells which required very much bigger doses before their migration ceased. The difference in response between these two main groups is in accord with the observations which have already been noted, based on histological studies of sectioned material.

Apart from the lymphocytes, a vast amount of work has been done on the irradiation of normal cells and tumours and it is generally agreed that the sensitivity of cells to X-rays is a function of their rate of growth. Thus, Ewing (1929) observes: "High metabolism of the tumour cells was early recognized as a prominent factor in radio-sensitivity. It is so nearly synonymous with rapid growth that one may usually judge of radio-

sensitivity by rate of growth." Similarly, Cowdry (1924) writes: "Inasmuch as the more reactive tissues have their molecules in the least stable form, these tissues will be the easiest to affect by X-rays. Thus, dividing cells in which the metabolism is very intense . . . are very easily destroyed. . . ." Osgood, Aebersold, Erf and Packham (1942), who incidentally found like Heineke that in irradiated marrow the lymphocytes and pro-granulocytes were equally sensitive, inclined also to the view that the chief effect of irradiation was to inhibit mitotic and amitotic division. Judged, then, from the standpoint of radio-sensitivity, the lymphocytes should be among the most actively growing cells in the body.

Toxic compounds

In addition to irradiation, numerous chemical substances have been used with a specific destructive action on lymphoid tissue. The 2-chloroethyl and 3-chloroethyl compounds are examples of such substances which have had considerable therapeutic application (Gilman and Philips, 1946, Goodman *et al.*, 1946). Kindred (1947) described in detail the action of these compounds on the haemopoietic system of the rat, and later (1949) of the dog

He found that in the rat "The degenerative changes of the hemopoietic organs as a whole resembled more closely those produced by roentgen rays than those caused by any other agents". He noted further that the changes were the same in adrenalectomized as in normal animals, and were not therefore due to stress reactions, effected through the adrenal cortex In the dog, he observed that the β-chloroethyl vesicants "exert a selectively toxic action on the nuclei of actively proliferating cells of the lymphoid organs and bone marrow. . . . The leukopenia of the peripheral blood followed the same pattern as was observed in the victims of the atomic bomb radiation in Japan, and in animals exposed to the atomic bomb radiation . . . at Bikini" Both in rat and dog the reticulum cells were much less susceptible than the lymphocytes, and in the rat Kindred actually thought that "the reticulum cells of the lymphoid organs are not directly injured by the agents".

Urethane was introduced by Haddow and Sexton (1946) and was first applied to the treatment of experimental tumours in animals. Subsequently (Paterson *et al.*, 1946, 1947) it was used in cases of leukaemia, both myeloid and lymphatic, in man. Unfortunately, detailed histological studies of the changes induced by urethane in lymphoid tissue have not yet been made.

Haemorrhage

Hellman has clearly realized the importance of treating the lymphoid tissues as a whole and reference has already been made to his basic data

Furth and Bigelow (1952) studied the cell content of lymph in rats and dogs at varying periods after exposure to massive doses of X-rays. They found that both in lymph and blood there was a precipitous drop in lymphocytes within 5–10 hours after irradiation, but that in addition large numbers of erythrocytes appear in the lymph. This is merely an exaggeration of a normal process (see p 324), but when it can lead to the presence of more than 1,000,000 erythrocytes in a c.mm. of lymph, it seems clear that this diversion of erythrocytes could be an important contributory factor in post-irradiation anaemia. It is tempting to explain the increased erythrocyte content of lymph as the result of damage to blood capillaries, with increased passage of red cells into lymphatics and their return to the blood via the main lymph ducts. If this is the case, then experiments such as those of Ross *et al.* make it clear that such damage to the blood capillaries may have an unexpectedly delayed onset, since it was only after 7 days that the thoracic duct lymph began to contain greatly increased quantities of erythrocytes.

It has also been suggested that post-irradiation anaemia may be due to increased destruction of erythrocytes (Barrow and Tullis, 1952), or to the development of a haemorrhagic state associated with thrombocytopenia (Jackson *et al.*, 1952).

Changes in the Lymphocytes and their Significance

Schrek (1947) made a careful study of lymphocytes from the rabbit thymus suspended in homologous serum, incubated aerobically at 37° C. and then irradiated. He described the formation of intranuclear vacuoles, which later discharged into the cytoplasm, leaving behind a dead pyknotic nucleus, and he concluded that the X-rays were accelerating a normal metabolic process, though without any evidence as to the nature of this process.

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(1953) found no evidence that they passed to lymph node, spleen, or liver. Unfortunately, in the experiments so far reported he has not examined the bone marrow.

Growth in the Absence of Bacteria

Glimstedt (1936), concerned with the effect of bacteria upon the lymphoid tissues as a whole, succeeded in raising 8 guinea-pigs under conditions in which bacteria were completely excluded. He found that the absence of bacteria seemed to have a harmful effect on the growth of the animals, which were subnormal in weight. This poor development was most marked in the lymphoid tissues, which also failed to develop germ centres. The total lymphoid tissue in the bacteria-free guinea-pigs was only about 25 per cent of that in the normal animal. Glimstedt concludes, therefore, that bacteria are the normal stimulus to the growth of lymphoid tissues especially, and to a definite though lesser extent of the body as a whole (cf. Gyllensten, 1954) He is also of the opinion that the germ centres are "reaction centres" as maintained by Hellman, since they do not develop in the absence of bacteria. Glimstedt's experiments are of great interest, but their interpretation meets with several difficulties. One-third of the animals (18 out of 57) which this investigator endeavoured to breed under sterile conditions died from no ascertainable cause. This is an extraordinarily high mortality and makes one suspect that the general condition of the animals must have been very poor. Later work, such as that indicating the possible synthesis of substances like vitamin B_{12} by intestinal bacteria, makes it easier to understand why aseptic growth might be fraught with unexpectedly serious consequences (Lewis *et al.*, 1942, Jukes and Stokstad, 1948).

This view is confirmed by the studies of Reyniers (1946), who states · "Although we have regularly reared guinea-pigs germ-free for 60 to 90 days and some as long as 8 months, *they were without exception malnurtured animals*" (the italics are ours) Most of the animals seemed to grow normally for about 30 days, but between 30 to 60 days a "generalized degenerative process" set in. The Lobund group were more fortunate with germ-free rats, though even here there was evidence of metabolic abnormalities The lymphoid tissues are singularly sensitive to malnutrition Unfortunately, no blood counts are given by Glimstedt, and no bone marrow findings at autopsy by Glimstedt or the other workers quoted

Complete lymphatic blockage

Blalock and his associates (1937) succeeded in producing complete blockage of the entrance of lymph into the circulation in 3 dogs In these 3 animals, lymphocytes and eosinophiles disappeared almost entirely

on the total amount of lymphoid tissue in the body. Working in Hellman's laboratory, Sjövall (1936)—who gives an excellent review of the earlier literature—has investigated the effect of anaemia on lymphoid tissue. This investigator subjected 47 rabbits to repeated bleedings, withdrawing from each animal about 1,000 ml. of blood over a period of 90 days. The mean weight of the total lymphoid tissues at the end of the experiment was 10·185 g., as compared with 12·283 g. in a control group. The red cells in the blood fell from 5,000,000 to 3,000,000 per c.mm., and the bone marrow showed greatly increased erythropoietic activity. Sjovall argues that if it is true that lymphocytes are continually being filtered out of the blood stream into the bone marrow, where they constitute stem cells for erythrocytes and granulocytes, then, under the condition of the experiment, there should have been a greatly increased demand for lymphocytes This increased demand ought to have been reflected in hypertrophy of the lymphoid tissues; instead, they actually diminished. At the same time, the blood lymphocytes fell from an average of 6,000 per c.mm. before the bleeding, to 3,500–4,000 after. This change in the level of the blood lymphocytes may not, however, be very significant (see Chapter 6) Wiseman (1931*b*) has shown that "the total number of lymphocytes circulating at any given time is not necessarily an index to lymphoid activity". As far as the weight of lymphoid tissue is concerned, Andreasen (1945) in a number of normal rats of varying ages dissected out and weighed the lymphoid organs, but found no correlation whatever between these weights and the number of lymphocytes in the blood. Apart from these considerations, Sjövall's interpretation of his results is open to criticism from other angles (see for example p. 424).

Fichtelius (1951*b*) followed up some earlier work of Ottesen (1948) by injecting into animals P^{32}, which was then incorporated in the DNA (desoxyribose nucleic acid) of the nuclei of developing white cells, and could be used to identify these cells subsequently after they had entered the blood. By this method, and making use of a simple technique (Fichtelius, 1951*a*) for the separation of blood lymphocytes, he showed that following the administration of P^{32} there was a biphasic appearance of radioactive lymphocytes in the blood, with two peaks around the 4th and 15th days respectively. The second peak afforded scope for obvious speculation, and in subsequent experiments Fichtelius (1953) succeeded in showing that *bleeding induced the second peak earlier than if the* animal had not been bled, and that this second peak was at its maximum about 4 days after the bleeding. The second lymphocyte peak (or "second top" lymphocytes, as Fichtelius termed them) might perhaps be interpreted as an increased production of lymphocytes in response to the haemorrhage. But in virtue of their P^{32} content, these lymphocytes ought to be easily identifiable when they leave the blood. Fichtelius

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had elapsed could it be directly attributed to the extirpation They estimated that about 90 per cent of the total lymphoid tissue of the body had been removed, a figure of the same order as that reported by Sanders and Florey (1940), but unlike these workers they found nothing to indicate a regeneration of this tissue.

Sanders and Florey (loc. cit) in two rats performed thymectomy alone, without any significant alteration of the blood lymphocytes. This is particularly interesting in view of the work of Kindred (1938, 1940), and of Andreasen and Christensen (1949), who considered the thymus as the most important lymphocyte producing organ. It seems clear from these extirpation experiments that however important the thymus may be, other lymphoid structures can adequately maintain the blood lymphocytes, and in fact Sanders and Florey concluded that in the rabbit the main source of the blood lymphocytes was the pancreas of Aselli It is evident from the experiments of Andreasen himself (1943) that there are difficulties in regarding the thymus as the main lymphocytopoietic organ, for he found that in starved rats the thymus atrophied more completely than the other lymphoid tissues, while on the resumption of feeding it regenerated at a much slower rate. In discussing his results, Andreasen states: "I wish to emphasize that while possibly the daily supply of lymphocytes to the blood is covered in part by the thymus, the increase in the lymphocyte count that takes place immediately after the discontinuance of a starvation period cannot be taken as an expression for the function of the thymus, as this organ at that juncture presents an extreme degree of atrophy."

The general tenor of all these extirpation experiments is in line with the observations of Andreasen (1945), who in a number of normal rats of varying ages dissected out and weighed the lymphoid organs, but found no correlation between these weights and the number of lymphocytes in the blood. The lack of this correlation, in view of his (1943) earlier observations about the variability of the lymphocyte content of lymphoid tissue, is not altogether surprising. But even if the lymphoid tissues were uniform in composition and in cell proliferation, there are still grounds for not accepting the blood lymphocyte level as a reliable index of lymphocytopoietic activity (see Chapter 6).

Splenectomy

Though the spleen contains only a small part of the total lymphoid tissue of the body, which would not therefore undergo serious diminution if it were removed, the literature dealing with the relation between the spleen and the other lymphoid tissues brings out certain features of interest. Whitney (1928), for example, after a review of the earlier literature, writes. "In general the opinion seems to be that following splenectomy there is a late lymphocytosis in the blood appearing four months to

from the blood stream; the animals rapidly lost weight and were killed as soon as it became obvious that they were going to die. The results indicate that the bulk of the blood lymphocytes reach the blood via the lymph stream, since obstruction of this stream leads to their almost complete disappearance. It would appear from these experiments that the number of lymphocytes which enter the blood directly, without the intermediation of the lymph, must be almost negligible. In other words, neither spleen nor bone marrow supply many lymphocytes to the blood

Direct Extirpation

The diffuseness of distribution of the lymphoid tissues, rendering complete extirpation impracticable, has made it very difficult to investigate their functions experimentally. However, Sanders and Florey (1940) evolved an ingenious technique for the operative removal (rats and rabbits) of what would appear to be the major part of the lymphoid tissue of the body, after preliminary vital staining by pontamine sky blue. They removed the spleen, thymus and most of the lymph glands, and in some cases Peyer's patches. After extensive lymphadenectomies the blood lymphocytes usually diminished somewhat, though not invariably. "In 2 rats the final level was actually a little above the original one, and even in a rat in which 93 per cent of the organized lymphoid tissue as well as that present in the spleen but excluding Peyer's patches was removed, the blood lymphocytes only fell to a quarter of their previous value"

Sanders and Florey (1940) further found that lymph glands which had been removed did not regenerate, but that there was marked compensatory formation of new lymphoid tissue in the liver (rat) or lung (rabbit); and they concluded that it was these new formations which helped to maintain the blood lymphocytes. That the lymphoid tissue was thus replaced, they interpreted as meaning that lymphocytes in large numbers were needed by the body Turner and Hall (1943) removed about one half of the organized lymphoid tissue of normal white mice, and found after 10 days that the remaining lymph nodes had increased by about 46 per cent in weight. They suggest that "some substance in the lymph reaching a lymph node normally maintains the size of that node. The substance being inactivated in this node cannot then influence the growth of the more centrally situated nodes."

Andreasen and Gottlieb (1946) performed, in rats and guinea-pigs, extirpation experiments similar to those of Sanders and Florey (1940), and paid special attention to the effect upon the blood As in the experiments of Sanders and Florey, both thymus and spleen were removed in addition to lymph glands. They observed a marked lymphopenia which lasted for 3–6 weeks, but on the basis of control experiments decided that for the first 24 hours or so this was non-specific, and only after this time

The experiments of Ensell and Yoffey (1955), in which a quantitative technique was used for the enumeration of the marrow cells, show that 40 days after splenectomy in the guinea-pig there is an increase—though not statistically significant—in the number of lymphocytes in the marrow, a finding which accords with the observations of Jordan and Robeson (1942), who reported the development of lymphoid nodules in the bone marrow of pigeons after subtotal splenectomy. However, in the experiments of Ensell and Yoffey there was no formation of lymphoid nodules in the bone marrow, nor anything which would indicate active lymphocytopoiesis in that tissue, so that the increase in the number of marrow lymphocytes was interpreted as being due to an increased uptake of these cells from the circulating blood. The possibility thus suggests itself that the spleen acts in some way as a brake on lymphocytopoiesis, on the uptake of lymphocytes by the marrow from the blood, or on both.

Lymphoid Tissue as a Defence Mechanism

Barrier Theory

It has been long held, mainly as a result of clinical observation, that the lymphoid tissues play an important part in the body's defence reactions We owe to Virchow (1860) the first precise formulation of the theory that the lymph nodes act as barriers. This author points out that in a lymph node " the elements lie crowded together like the particles in a charcoal filter, so that the lymph trickles out again on the other side in a more or less purified state ". Further, discussing the fate of pus which has entered the lymphatics, Virchow writes : " . . . it is manifest, that no pus corpuscle can pass a gland " He then proceeds to discuss the filtration of inanimate particles, using as his example tattooing, in which cinnabar or gunpowder was rubbed into a number of pin pricks, some of which must inevitably have punctured lymphatic vessels " . . . in the rubbing in a certain number of the particles find their way into the lymphatic vessels, are carried along in spite of their heaviness by the current of [illegible] deposit themselves in any way in the parenchyma of internal organs. No, the mass always settles in the nearest group of glands." Virchow illustrates this point with a section of an axillary gland from a soldier whose arm had been tattooed nearly 50 years previously The section happened to pass through a cortical vessel, around the ramifications of which there were to be seen masses of cinnabar. Of this, he writes : " None of it has

one year after the operation and persisting two to three years. There is a slight initial enlargement of the lymph nodes but it does not persist There is little if any increase in the lymphoid elements in the bone marrow." Krumbhaar (1922) believed that after splenectomy there might be actual hyperplasia of the marrow, and indeed throughout the literature one finds occasional though far from constant reference to post-splenectomy changes in the marrow suggestive of hyperplasia.

Loesch and Witts (1924) noted that following splenectomy or when all the large arteries or veins of the spleen were tied, there developed a severe anaemia, which reached its maximum one or two months after the operation. They also noted that there was enlargement and hyperplasia of the abdominal lymph glands, many of which had apparently become converted to haemolymph glands, and they inclined to the view that it was the erythrophagic function of these glands which was largely responsible for the development of post-splenectomy anaemia

As far as the clinical literature is concerned, one should perhaps draw a distinction between diseased spleens, and healthy spleens which have been removed because of trauma. Singer *et al.* (1941) reported on the haematological changes in 19 patients whose spleens had been removed on account of disease, at times ranging from 4 weeks to 15 years previously. In about half the cases there was a persistent absolute leucocytosis, involving mainly lymphocytes and monocytes. On the other hand Ek and Rayner (1950), reporting on 18 cases in which presumably healthy spleens had been removed after injury, found 14 years later that there was no sign either of lymphatic hyperplasia or raised blood lymphocytes. However, in 12 cases in which the marrow was examined, there appeared to be abnormally large numbers of erythroid cells In contrast, Ask-Upmark (1935) reported that after splenectomy the blood of patients might show departures from the normal for many years.

Palmer *et al* (1951) found that . " . . following splenectomy in the albino rat, the total leukocyte count increased approximately 100 per cent in 7 days, and remained significantly elevated for 70 to 90 days, after which the leukocytes returned to normal levels. This increase in circulating leukocytes was due to an increase in both neutrophiles and mononuclear cells." They found however that if 9 7 per cent of the spleen was left the leucocyte count remained normal, and they further noted that, following splenectomy in one partner of parabiotic rats, there was no leucocytosis When the spleen of the second partner was removed, leucocytosis occurred in both. Experiments such as these seem to suggest the existence of a humoral factor or factors through which the spleen may influence the lymphoid tissues and the bone marrow, and it will be recalled that this is a possible explanation of the benefits of spleen shielding in irradiated animals.

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penetrated farther than this spot ; even the next layer of follicles does not contain any. The particles are, however, so small, and the majority of them so minute in comparison with the cells of the gland, that they cannot at all be compared to pus-corpuscles. Now when such molecules as these are unable to pass, when such extremely minute particles cause an obstruction, it would be somewhat bold to imagine that pus-corpuscles, which are relatively large, could effect a passage."

The idea is then extended to cover malignant growths : "When an axillary gland becomes cancerous, after previous cancerous disease of the mamma, and when during a long period only the axillary gland remains diseased without the group of glands next in succession or any other organs becoming affected with cancer, we can account for this upon no other supposition than that the gland collects the hurtful ingredients absorbed from the breast, and thereby for a time affords protection to the body, but at length proves insufficient, nay, perhaps at a later period itself becomes a new source of independent infection to the body, inasmuch as a further propagation of the poisonous matter may take place from the diseased parts of the gland."

For many years the barrier theory held the field, and was not seriously challenged, so that McMaster and Hudack could write in 1935 . "Pathogenic bacteria carried in the lymph stream are often arrested in the glands through which this stream passes, with the result that infection travels no further."

Once the barrier theory, which sounds convincingly simple, had been accepted for lymph nodes, the concept of a defence mechanism spread almost imperceptibly to other lymphoid tissues, even though, in their case, the justification was not so obvious. In the case of the lymph nodes the flow of lymph through the sinuses and their reticulum may not constitute proof of the barrier theory, but it at least has the merit of making it sound quite plausible ; whereas in the case of the tonsils, Peyer's patches, and appendix no such structural basis for a defence function is to be found, and the hypothesis that these structures exercise a protective rôle rests on very unconvincing evidence. Nevertheless the idea gradually became prevalent that the tonsils and the other naso-pharyngeal lymphoid masses were somehow guarding the gateway to the alimentary canal—though exactly how they achieved this was never very clear—and it was not long before the appendix, Peyer's patches, and lymphoid nodules scattered through the intestine also came to be regarded as essentially protective in nature.

Extensions of Barrier Theory

In recent years, the defence theory has had two important extensions : the concept that the lymphoid nodules may constitute reaction centres

where noxious material is filtered out of the circulation; and the idea of lymph nodes as sites of antibody formation.

Reaction Centres

Hellman (1921) and Hellman and White (1930) believed that the lymphoid tissues protected the body against harmful substances circulating in the blood stream. This function was thought to be discharged by the reticulo-endothelial cells and also by the germ centres, which Hellman called "reaction centres" and believed to be regions where noxious material is filtered out of the circulation. This assigns to the lymph nodes a dual defence rôle. Not only do they prevent noxious agents which have entered the lymph from reaching the blood, but once they have entered the blood, the reaction centres quickly remove them. In other words, the reticulo-endothelial elements in the lymph sinuses act as filters to the lymph; the reaction centres, presumably, as filters to the blood.

Hellman and White (1930) gave to a number of rabbits repeated injections of killed cultures of *Bacillus paratyphosus B*. Subsequently the animals were killed, and the various lymphoid tissues examined. The most evident change was in the spleen, which was enlarged. There was considerable increase in the size of the germ centres in the Malpighian nodules, marked hypertrophy of the white pulp, and only a moderate increase in the red splenic pulp. The remaining lymphoid tissues, on the other hand, showed no such reaction. The intestinal lymphoid tissue underwent marked diminution; the lymph nodes generally showed no significant change; while the tonsils showed a slight tendency to increase in size, but nothing very definite. Hellman and White conclude, on the basis of the splenic changes, that there is increased activity of the lymphoid tissues in response to bacterial injections, and that the main site of the reaction is the germ centre. They identify the cells of the germ centres with the reticulo-endothelial apparatus and believe that these centres have both a simple defence function and the task of manufacturing antibodies.

Ehrich (1929*b*) in his earlier studies inclined to the same opinion. Working with rabbits, he made subcutaneous injections of staphylococci of low virulence, with resulting abscess formation. The regional lymph nodes underwent hyperplasia, and the blood showed a moderate lymphocytosis (Fig. 75). The germ centres were at first the seat of regressive changes, but at the peak of the lymphocytosis began to reappear. They then increased in number and size, while the blood lymphocytes returned to their normal level. Ehrich inferred from this that the germ centres are not concerned with lymphocyte formation, the implication being that they are reaction centres to the staphylococci or their toxins (cf. Sjovall

and Sjövall, 1930). Ringertz and Adamson (1950) and others have confirmed Ehrich's findings in regard to the hyperplastic changes in the regional nodes draining a focal infection.

Gyllensten (1954) found that in young guinea-pigs a subcutaneous

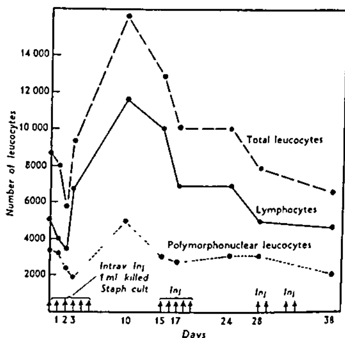


FIG 75—Effect of repeated intravenous injections of staphylococci on the blood leucocytes of rabbits
(From Ehrich, 1929*b*, Fig 1, p 364)

injection of a suspension of pseudomonas pyocyanea caused an increase in the weights of the regional lymph nodes and a highly significant increase in the number of secondary nodules. However, the same infection in newborn guinea-pigs, prior to the time when secondary nodules would normally have appeared, did not induce their premature formation.

Lymph Nodes as Sites of Antibody Formation

In 1898 Pfeiffer and Marx (cf. also Besançon and Labbé, 1898) showed that a single injection of heat-killed cholera bacilli into rabbits and guinea-pigs was followed by a definite increase in specific antibodies in the spleen, bone marrow, and lymph nodes, before any increase could be detected in the blood. The view that lymph nodes are a site of antibody formation would assign to them a dual rôle in defence: the holding up of bacteria and viruses reaching them from infected areas, and the elaboration of antibodies, which then pass into the blood. The nodes could conceivably

be instrumental in the production of a general immunity, capable of influencing not only the primary focus of infection but also any extensions from it

McMaster and Hudack (1935), reinvestigating the problem, demonstrated that agglutinin formation could take place in nodes, though their experiments threw no light on the actual cells concerned in the process. De Gara and Angevine (1943) showed after local injection of antigen a

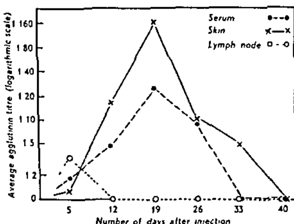


FIG 76—Average agglutinin titre of blood serum, of extracts from skin at the site of injection of the antigen, and of extracts of draining lymph nodes of 21 rabbits following one intracutaneous injection of pneumococcus vaccine

(De Gara and Angevine, 1943)

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transient appearance of antibody in lymph nodes, which never contained it in high titre. They did not however try to ascertain whether any particular cell type was concerned in the process. Ehrich and his collaborators have in recent years attempted to give a more specific answer to this question by studying carefully the changes in the popliteal lymph node of the rabbit and its efferent lymph following the injection of antigen into the pad of the foot

Cells involved in antibody formation

Ehrich and Harris (1942) first confirmed and extended the earlier experiments of McMaster and Hudack (1935) on the formation of antibodies in lymph nodes. As antigens they used either typhoid bacilli or sheep erythrocytes Antibodies began to appear in the efferent lymph of the popliteal node 2–4 days after injecting antigen into the pad of the foot, and their titre increased to a maximum by the sixth day. Lymphocyte counts of the efferent lymph showed a sharp increase in lymphocyte

output preceding and accompanying the rise in antibody titre, while in the lymph node itself there was a preliminary infiltration of granulocytes and monocytes, followed by hyperplasia of the lymphatic tissue, at first diffuse, but subsequently followed by large " so-called germinal centres, the latter lagging somewhat behind the rise in antibody titer".

Harris *et al.* (1945) repeated these experiments, but paid special attention to the antibody content of the efferent lymph. They first established the identity of the cells in this lymph, and concluded that 99 per cent of them were lymphocytes, monocytes being less than 1 per cent except in one instance where they may have risen to 2 per cent. After fractionating the efferent lymph (centrifuging at 1600 r.p.m for 10 min.) they found a considerably higher antibody titre in the lymphocytes than in the supernatant plasma, though if the suspension of lymphocytes in lymph was left for 24 hr before centrifuging, the difference in antibody content of cells and plasma became much less marked. They inferred from this that lymphocytes could readily part with their antibody; on the other hand further experiments showed that lymphocytes did not take up antibody even though incubated in plasma containing a high antibody titre These in vitro experiments were followed by in vivo experiments, with a similar result All these observations seemed " to indicate that lymphocytes are instrumental in the formation of antibodies". Ehrich, Harris and Mertens (1946) then proceeded to examine the antibody content of macrophages, and first showed that, in an experiment in which dysentery antigen was injected in the usual manner, the tissue at the site of injection contained only insignificant amounts of antibody, even though there were many granulocytes and macrophages present. On the other hand, the lymph nodes at this time contained considerable quantities of antibody.

They then injected antigen (dysentery and typhoid) into the peritoneal cavity and found that the peritoneal exudate contained first granulocytes, then predominantly macrophages. However, no antibody (agglutinins) was found in these cells, though the peritoneal fluid contained an appreciable amount, and the blood serum even more, on the average about twenty *times as much. With the elimination of the macrophage as either* a source or a storehouse of antibody it thus began to appear rather more probable that the lymphocyte was the important cell in antibody formation.

Dougherty *et al* (1944), Kass (1945), and White and Dougherty (1945) also showed that cells—again presumably lymphocytes for the most part —from minced lymph nodes were rich in antibodies. Chase, White and Dougherty (1946) reported that if adrenal cortical extract was given together with antigen, more antibody was produced than with antigen alone. They also noted that once immunity was established, injection of cortical extract could produce an increased antibody titre. However,

Eisen, Mayer, Moore, Tarr and Stoerk (1947) reported that "identical concentrations of serum antibodies and gamma globulin were found in adrenalectomized rats repeatedly injected during immunization with ACE and in similar animals not receiving ACE (extract of suprarenal cortex)"; a similar finding in cats was reported by Thatcher, Houghton and Ziegler (1948) It is pertinent in this connexion to quote the experiments of Andreasen, Bing, Gottlieb and Harboe (1948), who showed that in the rat extirpation of 80 per cent of lymphoid tissue was without significant effect on the normal range of serum proteins.

The Rôle of the Plasma Cell

Soon after McMaster and Hudack had begun their experiments in 1935, evidence began to accumulate from other workers that the plasma cell played an essential part in antibody formation (Bing and Plum, 1937, Rohr, 1938; Undritz, 1938). According to Fagraeus (1948) the administration of antigenic substances gives rise not only to the production of antibody, but is also a powerful stimulus to the formation of immature plasma cells from reticulum cells, especially in the splenic pulp; she found that as the titre curve of antibody levelled off, these became transformed into mature plasma cells, which were already past the stage of "greatest functional intensity" In experiments on the formation of antibodies in vitro, Fagraeus reported that (*a*) in the spleen the red pulp contained considerably larger amounts of antibody than the lymphoid follicles and that the capacity of the red pulp to form antibodies varied with its content of immature plasma cells, (*b*) that cultures of thymus, lymph nodes and bone marrow had a low antibody content (cf. Thorbecke and Keuning, 1953) Fagraeus correlated antibody globulin formation with histochemical changes in the plasma cells, namely, cytoplasmic basophilia and increased ribonucleotides.

If the work of Fagraeus is correct, there must be an essential difference between ordinary plasma globulin and antibody globulin, for after prolonged plasmapheresis there was no enlargement of lymph nodes or spleen, nor any increase in their content of plasma cells. Thorbecke (1954) has suggested that there may be no such thing as "normal" gamma globulin, and that it is only formed as part of the antibody response. Keuning and van der Slikke (1950) have further analysed the antibody-forming capacity of lymphocytes and plasma cells and have confirmed the view of Fagraeus about the essential rôle of the plasma cell, more especially of the immature variety, in antibody formation They immunized rabbits by intravenous injections of paratyphoid vaccine, and then prepared suspensions of splenic cells in which they separated the small from the large immature lymphoid cells Only the large cells were capable of forming antibody, whereas the small cells were not In this context it

is to be noted that Dougherty and White (1946) described in rabbits an increased formation of immature plasma cells in lymphoid tissue within a few hours after the injection of cortical hormone into rabbits. At the same time they noted that " many smaller lymphocytes may develop basophilic cytoplasm, thus giving to these cells the superficial appearance of plasma cells ".

Ehrich, Drabkin and Forman (1949) showed that in rabbits in which typhoid vaccine was injected into the foot pads, the popliteal lymph nodes increased in weight up to about the sixth day after injection, and that this weight increase was accompanied by a parallel rise in the content of desoxyribose nucleic acid (DNA), while the greatest increase in ribose nucleic acid (PNA) occurred between the fourth and sixth days after vaccine injection, when antibody formation was maximal. Histological examination of sections with methyl green (believed to demonstrate DNA) and pyronine (believed to demonstrate PNA) showed that " . . . during the first 6 days of the experiment the cellular reaction was chiefly one of plasma cells During the first 3 days plasmoblasts predominated; on the fifth and sixth days mature plasma cells were the prevailing cells. Most of the PNA was contained in the plasma cells." They concluded, therefore, that it is the plasma cell and not the lymphocyte which is responsible for antibody formation Ringertz and Adamson (1950), working with guinea-pigs, confirmed the intense plasma cell response evoked in the regional lymph nodes after repeated subcutaneous injection of antigen.

Reiss, Mertens and Ehrich (1950) prepared a suspension of cells from antibody-forming lymph nodes, and then added to this suspension the bacteria with which the animals had been immunized. Under these circumstances it could be readily observed that it was the plasma cells which agglutinated the bacteria, whereas the lymphocytes and the macrophages did not (cf. Moeschlin and Demiral, 1952, p. 413).

Antibody-forming cells in lymph

It should, however, be noted that plasma cells remain confined to the node and do not enter the efferent lymph Matsumura, Tanaka and Takenaka (1952) injected pertussis vaccine into the pad of the rabbit's foot, and 4 days later found that lymph draining the popliteal node contained 5 per cent of " plasmoid " cells. The name however is somewhat misleading, for as the authors themselves point out they were certainly not plasma cells, and from their account it appears probable that they are basophilic lymphocytes derived from reticular cells. Wesslén (1952), also working with rabbits, obtained thoracic duct lymph 3 days after the subcutaneous injection of living typhoid bacilli. The lymphocytes were centrifuged, washed in saline and then divided into two portions, of

which one half was at once lysed in distilled water, the other suspended in normal rabbit serum and cultured in a roller tube for 48 hours. The lysed lymphocytes did not contain specific agglutinin whereas the cultured lymphocytes did. Wesslén therefore concluded that while the cells in thoracic duct lymph do not contain antibody they are capable of forming it.

Wesslén also noted that 5–6 per cent of thoracic duct lymphocytes were of the large basophilic variety, and these, corresponding no doubt to the "plasmoid" cells of Matsumura, Tanaka and Takenaka would perhaps be the most likely cells to possess antibody-forming properties

Following the earlier observations of Chase (1951), some interesting contributions to the problem have been made by Harris and Harris (1951; 1954), Harris, Harris, Beale and Smith (1954), Harris and Harris (1955). They found that if they injected dysentery bacilli into the foot pad of a rabbit, and 4 days later teased the draining popliteal node in Tyrode solution, the intravenous injection of the cell suspension into another animal was followed by the appearance of agglutinins in the serum of the recipient rabbit The agglutinin titre reached its maximum by the third day, and began to decline by the fifth day. This result raised a number of problems. Thus, at the time of transfusing the suspension, the serum of the donor already contained a high titre of antibody and some antibody was also present in the cells obtained from the teased node Harris, Harris and Farber (1954) subsequently showed that the recipient rabbit developed agglutinins even when the donor cells of the teased popliteal node were obtained in as short a time as 10 min. after the injection of antigen into the pad of the foot; and it was also shown that the cells of the suspension could take up antigen in vitro. Evidently some of the lymph node cells are capable of fixing antigen very rapidly, but the experiments do not throw any light on the type of cell involved in this process.

The Employment of Fluorescent Antigen and Antibody

Additional evidence of a convincing nature is furnished by the experiments of Coons and his colleagues. Coons *et al.* (1942) showed that antibody labelled with fluorescein could be used as a specific histochemical stain Kaplan *et al* (1950) employed the technique to study the distribution of pneumococcal polysaccharides in the mouse and showed that as far as lymph nodes were concerned, the principal concentrations were in the reticulo-endothelial cells Small quantities of polysaccharide were found in some lymphocytes more especially in the medullary cords near the hilus. In later work, using fluorescent antigen, Coons, Leduc and Conolly (1953) were able to show that this was fixed by plasma cells in antibody-forming lymph nodes, but not by lymphocytes. A full account of the technique, and some further extensions of the work based upon it,

will be found in the recent papers by Coons, Leduc and Connolly (1955), Leduc, Coons and Connolly (1955), and White, Coons and Connolly (1955).

A stimulating discussion of the antibody problem, together with much fundamental information and some intriguing speculations, will be found in the monograph by Burnet and Fenner (1953). As far as the cellular aspect of the problem is concerned they conclude that "The antibody-producing mechanism is initiated by the entry of the antigen into the reticulo-endothelial system (macrophages)". From these cells modified antigen is transferred to "reticulum" cells or other relatively undifferentiated mesenchymal cells in the immediate vicinity of the macrophages. Under conditions inducing active antibody production these cells or some of them become active in protein synthesis and take on the staining qualities characteristic of plasma cells. They also suggest, though very guardedly, the possibility that the lymphocytes might be responsible for the production of small amounts of antibody over long periods.

The significance of lymphocyte proliferation in response to infection

One very interesting point emerges from the work of Ehrich and his collaborators. They derive the plasma cell from a specific plasmablast and not from the lymphocyte. The question then arises, what happens to the lymphocytes while all these changes are in progress? There is a definite lymphocytopoietic response, as distinct from the plasmacytopoietic, but with a very different time relation to the vaccine injection. Significant lymphocyte proliferation began on the third and fourth days, and was followed on the fourth and fifth days by the appearance of germinal centres. These reached their greatest activity on the ninth day, when PNA and antibody formation were already beginning to diminish.

It thus appears that one of the responses to an infection is the formation of lymphocytes in increased numbers and their passage into the blood via the lymph stream. Ehrich and Seifter (1953) tentatively suggest that they possibly function thereafter by undergoing dissolution and acting as trephocytes. This conclusion was based upon earlier work of Wagner and Ehrich (1950), who injected a vaccine into the foot pad of rabbits, and then found that the adenosinase activity of the popliteal node "rises with the activity of or lymphocytolysis in the germinal centres; it was not related to antibody formation, or the concentration of desoxyribose or ribose nucleic acid in the nodes". On this hypothesis it is difficult to understand why there is any need for lymphocytes to enter the blood stream. Lymphocytolysis and the passage of soluble protein and nucleic acid derivatives into the lymph and blood could take place quite readily by the breakdown of lymphocytes in the lymph node—as indeed Ehrich and Seifter (*loc. cit.*) themselves suggest.

Viruses and Lymph nodes

The relation of viruses to lymph nodes is discussed on p 289 but mention should be made here of the experiments of McMaster and Kidd (1937), designed to establish the development of antiviral principle for vaccinia in the regional lymph nodes of rabbits after local inoculation On the basis of these experiments they suggest that "the immunity conferred by clinical Jennerian vaccination may be largely of lymph node origin" However, their results would appear to be of questionable value in the light of later experiments by Yoffey and Sullivan (1939) on the passage of vaccinia virus through nodes These latter experiments indicate that in all probability vaccinia virus never remains strictly localized in the nodes, but escapes into the circulation within the first 12 hours.

General Considerations

Before considering in detail the experimental evidence bearing on the barrier theory, some general considerations must be taken into account. It has already been pointed out (Chapter 1) that only in birds and mammals do lymph nodes appear and become closely associated with lymphatic vessels The fundamental functions of the lymphoid tissues and the lymphatic vessels are apparently primarily independent of one another Their ultimate convergence must not be allowed to obscure this basic fact If the lymph nodes do finally come to act as barriers to the flow of lymph, they are not thereby discharging the primary function of lymphoid tissue—whatever that may be—but one which they have secondarily acquired

There is one aspect of the barrier problem which is usually completely disregarded In attempting to assess the defensive value of the lymphoid apparatus, *one must consider not only the nodes and other lymphoid* masses, but also the lymphatic vessels which are connected with them If we adopt this wider viewpoint, it becomes apparent that we are often dealing with a mechanism of doubtful utility, for even if one were to accept without reservation the view that the lymph nodes help to prevent the spread of infection or of malignant growths, it is obvious that the lymphatic vessels facilitate this spread. Any barrier action of the lymph nodes is more than offset by the dangerously rapid extension of disease that lymphatic vessels render possible. One has only to contemplate, for example, the case of a small epithelioma on the back of the hand with axillary metastases, a small septic wound with angry red lines running up to the axilla, or a carcinoma of the breast which has spread to the liver via the internal mammary lymph pathway or the lymphatics of the rectus sheath, to realize that, as far as barrier action is concerned, the patient would perhaps be better off had he possessed neither lymphatic vessels nor nodes.

Lymphatic Blockage in Local Inflammation

This aspect of the barrier problem has been emphasized by Menkin (1931; 1938), who made a careful study of what he called "lymphatic blockage". It appears from Menkin's work that in a focus of bacterial inflammation the exclusion of bacteria from the lymphatic vessels is much more to be desired than that they should enter and be transported rapidly to the regional nodes (cf. Dennis and Berberian, 1934).

Lymphoid Tissue of Alimentary Canal

Apart from the lymph nodes, the most notable collections of lymphoid tissue to which a protective function has been assigned are those associated with the alimentary canal—i.e. the nasopharyngeal lymphoid tissue, the appendix, Peyer's patches, and the solitary lymphoid nodules. The evidence that these collections of lymphoid tissue are not protective consists mainly in the fact that chronic infections frequently reside in the tonsils and in the intestinal tract; that acute infections, such as typhoid fever, have a special predilection for the lymphoid tissue of the bowel; and that, after such common operations as tonsillectomy, the general health of the individual is apt to improve and the incidence of throat infections to grow less. Statistical evidence on these issues—such as that presented by Kaiser (1922; 1932), by Hardy (1938), and by Glover and Wilson (1932) on the results of tonsillectomy—does not, however, offer convincing arguments against the protective function of the lymphoid tissues in question It is quite true that lymphoid tissue along the alimentary canal is most plentiful in early life and that during this period when it is most abundant it is frequently the site of infectious disease—a fact which may appear to militate against the conception that lymphoid tissue is essentially protective It is also true that infections tend to diminish in number as individuals grow older and as lymphoid tissue diminishes in quantity. But these two facts are not necessarily related. One may speculate that lymphoid tissue diminishes with maturity because at this period of life the risks of infection are not so great as in childhood, or because in the adult other defensive mechanisms have become better developed. One may, in fact, argue plausibly both for and against intestinal lymphoid tissue as a defence mechanism. But it cannot be too strongly emphasized that if it is a defence mechanism, it is one which breaks down with remarkable frequency. Furthermore, as far as the lymphocyte itself is concerned, we do not know how a defence function is exerted, and all the evidence which has accumulated in recent years has served to bring out this fundamental fact more forcibly than ever before.

Filtering Capacity of Lymph Nodes

Since the observations of Virchow, a very extensive literature has grown up around the problem of filtration by lymph nodes. (See Besançon and Labbé, 1898; Oeller, 1928; and Hellman, 1930.) Discussion has centred mainly around the filtration of substances from lymph, although Schulze (1925) has raised the question of filtration of noxious substances from the blood through "stomata" in the postcapillary veins of lymph glands. From a survey of the literature, however, it is clear that the spleen, bone marrow, and liver are the organs primarily responsible for the removal of bacteria from the blood, whereas the rôle of the lymphoid tissue in this respect is of minor importance (Wyssokowitsch, 1886, Bull, 1915).

As far as the lymph is concerned, it has been held that the nodes provide two main types of filtration. The first is of a simple mechanical type; the second involves, in addition, a biological reaction on the part of the phagocytic elements in the node, chiefly the reticulo-endothelial cells Even after a great deal of indirect experiment and speculation, opinions as to the combined efficiency of these two types of infiltration —normal nodes alone being under consideration—vary from assertions of complete effectiveness to statements of utter uselessness.

Inanimate Particles

In Chapter I mention has been made of experiments on the perfusion of lymph nodes with inert particles (India ink and acacia-graphite mixtures) It will be convenient here to amplify certain points which were not previously dealt with

Fig 77 is a drawing of a popliteal node injected with a dilute solution of India ink The injecting mass was delivered through a cannula in one of the large trunks along the small saphenous vein. The injection pressure was 20 mm Hg This is less than the pressure which can be developed in the same vessels when the muscles of the leg are stimulated electrically The injection in such an experiment enters the node through a number of afferent trunks, which pierce the capsule obliquely and open into the marginal or cortical sinus. This sinus is not a channel but a large bowl-shaped lake, bounded on the outside by the capsule of the node and on the inside by the lymphocytic parenchyma. The lake is traversed by fibrous trabeculae and blood vessels and, like all the sinuses, is crossed and recrossed by a fine mesh of reticulum, most important for the filtering function of the node From the cortical sinus irregular but numerous cleft-like channels, the intermediary sinuses, pass between the masses of lymphoid tissue toward the hilus of the gland, where they rejoin the cortical sinus to form the efferent lymph vessel. It is fortunate, from an

experimental point of view, that this efferent vessel is usually single at the hilus, thus permitting complete collection of all lymph passing through the node.

The architecture of the sinuses has a very direct relation to the filtering capacity of the node. Not only are the sinuses crossed and recrossed by the reticulum, but their walls are incomplete wherever lymphocyte growth is active (Drinker, Wislocki and Field, 1933). This means that

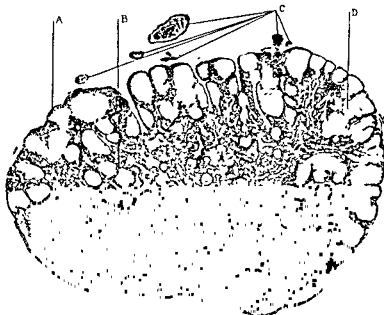


Fig 77 —Camera lucida drawing of a section of the popliteal lymph node of a dog injected through the afferent lymphatics with a dilute suspension of India ink

A, cortical sinus, *B*, intermediate sinus, *C*, afferent lymphatics in the capsule, *D*, apparent spaces, in reality collections of lymphocytes not permeated by ink and consequently of low opacity × 9

(From Drinker, Field and Ward, 1934, Fig 1, p 394)

at many points, particularly on the inner surface of the cortical sinus, the lymph may run out into the lymphoid tissue.

Nordmann (1928), as a result of study of sections of nodes, arrived at the conclusion that lymph traverses a node in three ways Most of it reaches the efferent vessel without leaving the marginal sinus. A second fraction, much smaller, finds its way via the intermediary sinuses; and a third, very small part drifts from the marginal sinus into the lymphoid tissue and finds its way back into the main stream, either by rejoining the marginal sinus or by reaching an intermediary sinus. Maximow (1931) adds another possibility, which we have not encountered; namely, an

occasional endothelium-lined lymphatic which goes directly through the [illegible] infrequent and must represent

deposited on the walls and reticulum of the intermediary sinuses. The masses of lymphoid tissue are but slightly penetrated by the ink, but the barrier to the entrance of the injection is constituted, not by an intact

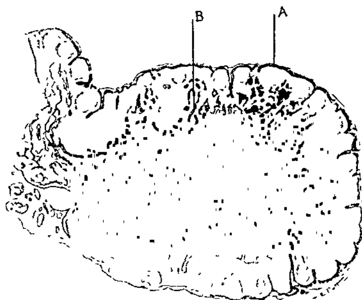


Fig 78—Camera lucida drawing of a section of the iliac lymph node taken from the dog which provided the popliteal node of Fig 77 *A*, cortical sinus, *B*, intermediate sinus × 13

(From Drinker, Field and Ward, 1934, Fig 2 p 396)

sinus wall, but rather by the closeness with which the lymphocytes are packed together, and by the density of the reticular fibre framework at the periphery of the lymphoid nodule

Fig 78 is a camera lucida drawing of an iliac lymph node taken from the dog which provided the popliteal node seen in Fig. 77. It is clear that, in spite of the filtering mechanism, a fair quantity of ink has passed through the popliteal node to reach this iliac node The same phenomenon was repeatedly observed both with India ink and acacia-graphite suspensions, though the total time during which the suspensions were perfused through the popliteal node was never more than 10 minutes.

In the iliac node, the marginal sinus is not entirely filled, and the ink has flowed into many intermediary sinuses.

As a result of numerous perfusions, it seems clear that lymph entering the marginal sinus flows as readily through the intermediary sinuses as it does through the cortical sinus. The entire arrangement, from the point of view of mechanics, appears excellent for filtration Lymph flowing in through a number of narrow channels and under a very definite head of pressure finds itself in a huge space with an enormous number of wide and irregular paths leading to the hilus vessel. The flow is instantly slowed and the driving head of pressure practically lost. Not only are the sinuses in the node a perfect settling chamber, but the reticulum which they contain furnishes a multitude of baffles, which again slow down the lymph flow and make it easy for the phagocytic cells composing the reticulum to perform their function

Red Cells

In a second group of lymph node perfusion experiments, the perfusing fluid was autogenous, heparinized plasma, diluted with physiological salt solution until the protein content was approximately 1 per cent. To this artificial lymph, red corpuscles were added to give a count in the neighbourhood of 25,000 per c.mm. In these experiments—as in those with India ink just described—the blood supply to the nodes was intact, the afferent and efferent lymphatics were cannulated, but the node itself was not disturbed.

In a typical experiment (Drinker, Field and Ward, 1934), the perfusion was continued for 2 hours and 5 minutes at a pressure of 16 to 20 mm Hg, 9 ml of perfusate ran in, and 7 6 ml were collected from the cannula in the efferent lymphatic The total effluent was collected as 13 separate specimens. Of this number, 9 contained no red cells, 3 contained 200 red cells per c mm., and one contained 400 red cells per c mm The filtration was therefore, fairly complete. If, however, the node was massaged, even very gently, the effluent at once began to contain red cells in large numbers.

On microscopic examination of the node, red cells were found in both cortical and intermediate sinuses, but were most numerous in the latter. Many were phagocytized by reticulo-endothelial elements, but the greater number lay in closely packed masses scattered through the reticular meshwork of the intermediate sinuses.

As against these experiments one must set those of Courtice, Harding and Steinbeck (1953), who, working with cats, found that red cells which were injected intraperitoneally were removed through the lymphatics of the diaphragm, and finally reached the right lymph duct in concentrations of the same order as those in which they were found in the blood. These

cells had apparently passed through mediastinal lymph nodes, but despite the fact that the macrophages in the latter were engorged with red cells, the bulk of these had traversed the lymph nodes with ease. There was in these experiments no question of lymph node massage other than that provided by the normal movement and pressure changes in the thorax. Hughes, May and Widdicombe (1955) found in the rabbit that 3–30 per cent of red cells passed through the popliteal node after injection into an afferent lymphatic. It may therefore well be that there are important species differences. In the dog, the animal used by Drinker, Field and Ward in their filtration experiments, the trabecular network in the lymph sinuses is particularly well developed.

It is possible also that the peritoneal cavity may present a rather special case, for blood which has been introduced into it is readily absorbed via the lymphatics into the blood-stream in many species of animals, including man and dog.

Bacteria

Schulz, Warren and Drinker (1938) noted that type III pneumococci which had been dropped into the nose of rabbits could be recovered from

TABLE 37

Passage of pneumococci through nasal mucous membrane of rabbits *

No	Weight	Temperature	0·5 *hr*		1·0 *hr*		1·5 *hr*		2·0 *hr*		2·5 *hr*		3·0 *hr*		3·5 *hr*		4·0 *hr*		
			C	B	C	B	C	B	C	B	C	B	C	B	C	B	C	B	
	kg	°*C*																	
20	1·9	39·0	0	0	0	0	+ +	0	+	0	0	0	+	0	+ +	0	+ +	0	
21	2·6	40·0	0	0	+ +	0	0	0	+ +	0	0	0	+	0	+	+	+	0	Flow scant
22	1·6	36·0	0	0	0	0	0	0	0	0	0	0	+	0			+	0	" "
23	2·7	37·2	0	0	0	0	0	0	+	0	+ +	0	+	0	+	0	+ +	0	
24	2·0	38·0	0	0	0	0	0	0	+	0									
25	2·2	38·2	0	0	+	0	0	0	0	0	0	0	0	0		0		0	Lymphatic dilated above node
26	2·2	38·1	0	0	0	0	+	0	0	0	+ +	0	+ +	0	+	0	0	0	
27	2·1	38·0	0	0	0	0	0	0	+	0	Died								
28	2·0	37·7	0	0	0	0	0	0	0	0	+	0	Died						
29	2·0	38·5	0	0	0	0	0	0	0	0	+	0	+	0	+	+	0	0	Flow scant

* This table shows the number of organisms found in cervical lymph and in blood at varying intervals following the instilling of 2 to 3 ml. of rabbit-virulent type III pneumococcus culture into the noses of normal rabbits. +, *1 to 5 organisms per half hour sample*; + +, *5 to 15 organisms*. *C*, cervical lymph; *B*, blood.

From Schulz, Warren, and Drinker (1938, p. 255)

the cervical lymph, after passage through the superior deep cervical node, often within one hour. These experiments, however, are open to the objection that in the massage of the cervical duct necessary to obtain lymph

samples in the rabbit (see Yoffey and Drinker, 1938) the node itself must undoubtedly have been massaged at times, a manoeuvre that would dislodge particles from the sinuses of the node. This, however, may not be a very serious objection, since there is no doubt that even in the normal animal the cervical node is continuously exposed to a gentle massage by reason of head movements and contraction of overlying muscles. Furthermore, the type III pneumococcus is notoriously virulent for the rabbit, and one has to consider the possibility of extremely virulent organisms passing through a node where less virulent ones might be held up—a contingency of which Cornet (1914) believes the reverse to be true; namely, that a virulent organism is more likely to be held up than one of lesser toxicity.

Drinker, Field and Ward (1934) performed experiments to test both the *filtering efficiency of a single node—the popliteal—and the filtering* efficiency of the complete lymphatic pathway from the leg below the popliteal node to the point where the thoracic duct empties into the blood In one experiment of the first group, the popliteal node was perfused with an undiluted serum-broth culture of a strain of haemolytic streptococcus, known as *Streptococcus I.* The culture contained 600,000,000 colonies per ml. The perfusion pressure was 34 mm. Hg. In 1 hour and 20 minutes, 5 ml. of the culture ran into the node and were collected from the efferent lymphatic. Cultures of the entire effluent showed 4,500,000 colonies per ml Filtration, therefore, was 99 per cent complete

A section of this popliteal node (Fig. 79) shows that the distribution of bacteria, from a mechanical point of view, is no different from that of India ink. (Compare with Figs 77 and 78.) In Fig. 79 part of the cortical sinus is seen just beneath the capsule. The black material in this sinus and in the Y-shaped intermediary sinus consists of masses of streptococci, both free and attached to cells. Fig 80 shows part of the marginal sinus just above a dense collection of lymphocytes into which the organisms have not penetrated This, however, is not *invariably the case* Regions may be found where the lymphocytes are quite solidly packed, but where large mononuclears, or occasionally polymorphonuclear cells containing cocci may be observed here and there.

When a gland is given such a huge dose of bacteria as in this experiment, blood cultures occasionally become positive, and this even after the precaution has been taken of tying the thoracic and right lymphatic ducts and both subclavian veins. The explanation may reside either in the migration into blood capillaries in the node of phagocytes containing streptococci still capable of growing, or in the entrance of bacteria into the blood through lymphaticovenous communications in the abdomen. Fig. 81 shows a capillary in the loose tissue just outside the capsule of the perfused node. In addition to red cells, there are present a number

of white cells containing microörganisms. The possibility of bacteria leaving a lymph node in phagocytes, which then enter the blood, introduces further complications in the interpretation of experiments such as those of McMaster and Hudack (1935) on the formation of antibodies in lymph nodes; *for in these experiments, the basic assumption was that bacteria*



Fig [illegible]

(*From Drinker, Field and Ward* 1934, plate 20, Fig 1, p 405)

which reach a node do not travel any further, either in the efferent lymph or through the medium of phagocytes.

There remains to be considered another possible channel of exit for bacteria in lymph nodes. Schulze (1925) believes that the post-capillary veins of lymph nodes possess "stomata", which afford a direct communication between blood and lymph, and that through these stomata noxious substances leave the blood stream and enter the node, where they

are then dealt with by the nodal tissues. If these stomata really exist, however, movement might also occur in the reverse direction, and micro-organisms might enter the blood capillaries from the node, provided some



FIG 80 —[illegible]
cocci [illegible]
cellula[illegible]
not ye[illegible]
(From Drinker, Field and Ward, 1934, Plate 30, Fig 2, p 405)

force could be found which would develop a current into the capillaries In the spleen, such a force is provided by the smooth muscle in the capsule and trabeculae. It is not easy to see just how a lymph node can retain structural integrity if it possesses open blood capillaries and yet no power

of rhythmic contraction which can be counted on to drive plasma and cells back into the blood vessels, and so clear the node of the excess transudate which must steadily accumulate. Furthermore, if the capillaries in the popliteal node are open, then lymph collected from an afferent vessel ought to contain much less protein than that from the efferent side. This is not the case. Protein concentrations in afferent and efferent lymph are identical.

The second group of experiments of Drinker and his associates referred

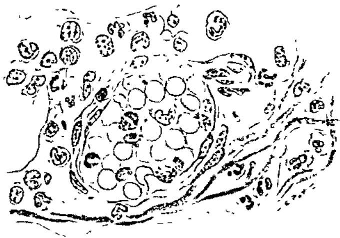


FIG 81 —Camera lucida drawing of a capillary in loose tissue just outside the capsule of a popliteal node (dog) perfused with streptococci White cells containing organisms are clearly shown

(From Drinker, Field and Ward, 1934, Plate 30, Fig 3, p 405)

to above (1934) was devised to test out the filtering efficiency of the lymphatic pathway as a whole, from leg to thoracic duct. The following is a typical example: At the lower end of the pathway, an afferent lymphatic of the popliteal node of a dog was cannulated in the usual manner, but the efferent vessels were left untouched At the other end of the pathway the thoracic duct was cannulated. To make sure that no lymph would enter the blood via the right lymphatic duct, this duct was tied and, as a final precaution, both subclavian veins were tied, central to the observed lymphatic entrances The perfusing fluid was an undiluted serum culture of a strain of haemolytic streptococcus, *Streptococcus I*,

delivered at a pressure of 20 to 40 mm. Hg. At the close of the experiment, 3 ml. of a 2 per cent solution of trypan blue were introduced into the perfusion cannula This appeared promptly in the thoracic duct lymph, and both the popliteal and iliac nodes were deeply and uniformly stained, so that there was no question of there being any obstruction to the lymphatic pathway from perfusing cannula to thoracic duct. Table 38

TABLE 38

Appearance of streptococci in insignificant numbers in thoracic duct lymph of dog after perfusion through popliteal and iliac lymph nodes *

Time min.	*Perfusion inflow ml*	*Perfusion inflow per min ml.*	*Thoracic duct lymph Colonies per ml.*
11 40 a m			Control specimen sterile in 1·0 ml.
0–13	2 8	0 22	Sterile in 1 0 ml
13–29	3 0	0 19	" " " "
29–43	2 0	0 14	" " " "
43–56	2 0	0 15	" " " "
56–71	2·0	0 14	*Sterile in 1 drop.* *Streptococci found in 1 0 ml*
71–88	2·2	0·13	" " " " " " " " "

* The perfusate, an undiluted serum culture of a strain of hemolytic streptococcus, Streptococcus 1, contained 250,000,000 colonies per ml at the close of the perfusion

From Drinker, Field and Ward (1934, p 402).

gives the results. In this experiment, in which thoracic duct lymph was used to assess filtration, it is clear that a high degree of filtering efficiency obtained. In another experiment, in which the perfusion pressure, instead of 20 to 40, was 40 to 50 mm Hg, a fair number of streptococci appeared in the thoracic duct lymph.

One important point emerges from these thoracic duct experiments. It has long been a question as to whether lymph leaving a region of subcutaneous drainage can reach the blood stream without passing through a lymph node. In this group of experiments, it is significant that the thoracic duct lymph never showed an immediate deluge of cocci, such as might be expected if nodes were short-circuited by vessels passing around them. Apparently, lymph from peripheral regions does not reach the blood until at least a single node has been passed. This observation we have further been able to confirm from numerous dissections of the cervical lymphatic pathway, in which a number of lymphatics converge to the superior deep cervical node, from which the main cervical duct emerges. In the course of these dissections we have never seen a lymph vessel pass by the node without entering it.

On the whole, these experiments indicate that lymph nodes possess a

fair degree of efficiency in filtering out bacteria which reach them through the lymph stream. This filtration may, however, break down when the number of bacteria reaching the node becomes excessive, when the lymph pressure becomes too high, when the organism is unusually virulent, when the node is subjected to massage, or when the bacteria are ingested by phagocytes which then migrate into blood vessels (cf. Hughes, May and Widdicombe, 1955).

The lymph pressures employed in these perfusion experiments varied up to 50 mm. Hg. It has been shown that the large afferent lymphatics in the leg of the dog will support pressures as high as 81 mm. Hg (Field, Drinker and White, 1932)—pressures obtained as a result of sterile inflammation of the foot. In an afferent lymphatic just below the popliteal node—one of the vessels used in these perfusion experiments—pressures of 50 mm Hg have been observed during repeated passive motion of the foot. It is thus clear that in normal animals, moving about actively, lymph pressures equalling those employed in these perfusion experiments may occur. Furthermore, the volume of the perfusate was not excessive. Measurements of the flow of lymph below the popliteal node in walking and running dogs (White, Field and Drinker, 1933; Weech, Goettsch and Reeves, 1933) show that rates of flow such as were employed in the perfusion of bacteria are not abnormal.

In a quiescent dog, the flow of lymph from such vessels soon becomes negligible and the lymph pressure falls to zero. If one considers the degree to which lymph nodes might filter organisms under actual conditions of disease, it is apparent that they would be subjected to a much less severe task than that imposed in the above experiments Given a cellulitis of the foot, the dog is ordinarily quiet and a heavy flow of lymph does not occur until swelling becomes extreme. It is also certain that lymph nodes in actual disease would rarely be confronted with such a deluge of organisms as was placed in the lymphatics in these experiments.

Viruses

Thus far we have considered the filtration through lymph nodes of inanimate particles, of red cells and of bacteria. When we turn to the viruses, the evidence suggests that we may be facing an entirely new set of conditions. In the experiments about to be described (Yoffey and Sullivan, 1939), no direct perfusions were performed, as with streptococci. But from the dye experiments of Yoffey and Drinker (1938), it had already been established that lymph from the nose and pharynx does not reach the main cervical lymphatic duct without passing through at least one node The basic procedure, therefore, of producing a virus lesion in the nose and then examining the cervical lymph below the node, is essentially the same as artificially perfusing the node, but with the added

advantage that lymph pressure and rate of flow are determined by conditions within the animal

If vaccinia virus is dropped into the nose of a rabbit, it cannot be detected in cervical lymph within 9 hours. Evidently the virus does not immediately pass through the nasal mucous membrane and enter the lymph, as is the case with dyes and proteins of low molecular weight. Twelve hours after the nasal instillation of virus, however, and up to 7 days thereafter (experiments were not carried beyond this period), virus can practically always be found in the cervical lymph. In other words, for 7 days after its administration a continuous stream of virus passes from the primary focus of infection in the nose through the cervical lymph duct into the blood. Now this virus cannot have entered the lymph without passing through at least one node, and the conclusion seems inescapable that the cervical lymph node does not constitute an effective barrier to the passage of virus. Since there is no obvious difference in structure between cervical and other lymph nodes, it seems likely that this relationship holds good for all nodes, particularly in view of the mechanism of fixation by lymphocytes whereby the virus is transported (see p. 399) This likelihood is further strengthened by the fact that virus was frequently found in thoracic duct lymph, to reach which the virus-containing lymph must have passed through one or more nodes (Drinker, Field and Ward, 1934) either in the limbs or in the abdomen; and since thoracic duct lymph flows spontaneously, without the massage necessary to secure cervical lymph, the possibility of virus—otherwise potentially fixed in the node—being liberated by nodal massage is eliminated. We may, therefore, fairly regard the finding of virus in thoracic duct lymph as further confirmation of the idea that lymph nodes do not constitute an effective barrier to the passage of vaccinia virus. The explanation of this phenomenon and some possible implications are discussed in Chapter 7.

Granting the passage of vaccinia virus through the lymph nodes, the next question to be considered is whether the virus is at all affected by this passage. There are theoretically three possibilities; the virus may pass through unchanged, it may be attenuated or even destroyed, or it may multiply or increase in virulence. Experiments of McMaster and Kidd (1937) throw light on this point. Suspensions of vaccinia virus were injected near the tip of the shaved left ears of 6 rabbits and 4 hours later—to allow time for some of the virus to reach the regional lymph nodes—the ears were amputated. Although the primary focus had thus been removed, it was found that during the next 3 days virus appeared to increase within the cervical lymph nodes; by the fifth day, less virus could be demonstrated, still less on the seventh, and none on the eleventh day.

Evidently, then, the cervical lymph nodes of the rabbit permit, for

some 3 days, the ready growth of vaccinia virus which has reached them; while the virus may still persist in the node for several days after, at least, and possibly even for many months (cf. Olitsky and Long, 1929).

The question at once arises whether other viruses may undergo dissemination in a similar manner. What, for instance, is the position in regard to the "neurotropic" viruses? This problem is further discussed in Chapter 7.

We are now in a position to sum up the experimental evidence on the filtration of lymph nodes. Inanimate particles are held up in nodes to a considerable extent, though by no means completely. Red cells may at times be completely retained, but on occasion they may pass through the nodes in large numbers with apparent ease. Bacteria are fairly efficiently filtered out, except under the conditions which we have already discussed. Viruses, on the other hand, if our experience with vaccinia is a reliable criterion, are filtered out very inefficiently. In fact, as far as viruses are concerned, the lymph node may be a source of danger rather than of safety, since it can actually serve as a centre first for the proliferation of virus, and subsequently for its diffusion throughout the body.

Blood-lymph Barrier

Passage of Bacteria and Antibodies from Blood to Lymph

In discussing the defence rôle of the lymphatic apparatus, we have so far considered two possibilities, that bacteria from a focus of infection may either enter the lymphatics and be held up in the sinuses of the regional nodes, or that they may enter the blood and be filtered out in the so-called reaction centres. In addition to these two processes, bacteria which are present in the blood may enter the lymph stream after passing into the tissues. This has been clearly shown both for thoracic duct lymph (Drinker, Enders *et al.*, 1935) and for lymph from the neck and limbs (Field *et al.*, 1937). In addition, these experiments contributed information on the antibody content of lymph, which must also be taken into account in assessing the defensive value of the lymphatic apparatus. The important fact emerges that while bacteria may readily pass from blood to lymph, antibodies will not do so in quantities sufficient to sterilize lymph. Consequently, bacteria either in lymphatic vessels or in the lymph sinuses of nodes are in a fluid of low bactericidal power. The significance of this, from the standpoint of defence, is obvious.

In recent years a number of protein [illegible]

..

active dyes such as T1824 (Evans blue) and brominated trypan blue, as

also halogenated plasma proteins, pass rapidly from blood to lymph, and in anaesthetized animals a maximal concentration in thoracic duct lymph is attained in 3–4 hours. The dyes are apparently bound to the serum albumin, which because of its smaller molecular weight enters the lymph more rapidly than globulin. These results have been confirmed by Krieger *et al.* (1950) who showed that human iodinated serum albumin, after intravenous injection into dogs, appeared more rapidly in thoracic duct lymph than did iodinated plasma proteins (cf. Wasserman and Mayerson, 1951; Abdou, Reinhardt and Tarver, 1952). This problem is further discussed in Chapter 2.

Lymphatic blockage and susceptibility to infection

The ability of microorganisms to flourish in lymph becomes further emphasized when we consider the phenomena of lymphatic obstruction These are discussed at length in Chapter 8. Briefly, it may be stated here that in a limb in which there has been induced chronic lymphatic obstruction and lymphoedema there is greatly increased susceptibility to infection. Lymph and oedema fluid from such a limb form an excellent culture medium for bacteria (Drinker *et al*, 1935).

Functions of Lymphoid Tissue in Health

Difficult as it may be to understand the rôle of lymphoid tissue in disease, the problem becomes even more perplexing when we consider the function of lymphoid tissue in health. The total amount of lymphoid tissue in the body, as pointed out in Chapter 1, is probably in the neighbourhood of 1 per cent of the body weight. What is the function of this "silent" lymphoid organ in persons who are suffering neither from infectious nor from neoplastic disease? Are we to assume that this lymphatic tissue is present throughout life merely on the offchance that at some time or other it may have a defensive role of doubtful utility, or can we assign to it an alternative function? Several such functions have been suggested, but the one best established is the continuous formation *of lymphocytes. The main problem of consequence* in a consideration of normal lymphoid tissue becomes, then, the problem of the lymphocytes which it is regularly producing in such large numbers.

It has been held that even in health lymph nodes are not bacteria-free, and that, through "physiological defects" in the skin and mucous membranes, a small number of organisms are continually entering the *lymphatics and being filtered out in the nodes* Adami (1910) elaborated this idea for the gastro-intestinal mucosa. He believed that a process of "subinfection" was constantly taking place. A scraping or swab from the surface of the nose or pharynx contains a fair number of leucocytes,

in many of which bacteria are to be found. Most of these leucocytes undergo dissolution or are swept away in the mucous secretions and saliva, but some find their way back into the epithelium. Nicholls (1904) showed that the intestinal lymphoid tissue of animals contained varying numbers of bacteria, most being found in the rabbit. Bacteria-containing leucocytes may be arrested in the intestinal wall, or may then pass to the mesenteric lymph nodes or to the radicles of the portal vein, and so become lodged in the liver. On careful microscopic examination, Adami found numerous minute granules in mesenteric nodes and liver cells which, at first sight, resembled pigment granules, but which he was convinced were the remains of disintegrated bacteria. Bloomfield (1915) endeavoured to culture organisms from 7 apparently normal nodes In 2 of these, he obtained organisms which he believed to be correlated with saprophytes on the body surface. They were aerobic, avirulent for rabbits, guinea-pigs and mice, and gave no immunity reactions with the patients' sera Although in 5 of the 7 nodes no organisms were found, Bloomfield suggested that saprophytic organisms, identical with or closely related to those on the body surface, were frequently filtered out, and perhaps even constituted a more or less constant flora of lymph nodes.

Many scattered references are found throughout the literature to the presence of bacteria in apparently normal lymphoid tissue and in other tissues Most of the work is extremely speculative, and experimental confirmation or disproof of the conclusions is so difficult that it seems to us an attitude of healthy scepticism is indicated. As Zinsser and Bayne-Jones (1937) observe, " Studies by many other workers, . . . and studies in our own laboratory show that organisms very similar to these strains [the large heterogeneous group of the diphtheroid bacilli] can be isolated from the skin, from the lymph nodes of healthy and diseased people, from ascitic fluid in various conditions, and from supposedly sterile tissues They are frequently present in the nasal mucus and in the throat, and are so ubiquitous that any association of them with specific disease must be very conservatively approached " It would be absurd to argue, because bacteria are often found in the intestinal mucosa or in the liver, that these structures are therefore not discharging their normal functions. Similarly, in spite of the occasional presence of bacteria in lymphoid tissues, the production of lymphocytes is as much a normal function of this tissue as is the formation of digestive juices a function of the intestinal mucosa, or the formation of bile a function of the liver.

Yet so strong is the hold which the barrier theory has taken that even lymphocyte production is looked upon as somehow connected with defence. There is perhaps a superficial analogy between the lymphocytes and the granular leucocytes. The latter also are being formed and are entering the blood throughout life, yet it is only when the emergency

arises that their defensive anti-bacterial functions are called into full action. However, granulocytes are capable of active phagocytosis when occasion requires. The granules in their cytoplasm are the visible outward sign of the elaboration of specific chemical substances which are also of value in combating bacterial products In further support of the defence idea it has been suggested (Bunting and Huston, 1921) that the lymphocyte may play a part in "affixing" toxins, but the evidence for this has been unsatisfactory (e.g. Moor and Newport, 1939); and the use of fluorescent antigen (Kaplan *et al*, 1950) has in fact shown quite convincingly that this is not the case on any appreciable scale.

It may be convenient here, before discussing them in detail, to list the functions that have been suggested for lymphoid tissue in health. There are six of them: (1) lymphocyte production; (2) metabolism and transport of protein and fat; (3) vitamin storage, (4) elaboration of internal secretion; (5) destruction of red cells; (6) the provision of nucleoprotein and other substances as adjuvants to cell growth. In view of the close association between the lymphocytes and the reticulo-endothelial cells in most lymphoid tissues, it is often difficult to dissociate the functions of the two cell types. The reticulo-endothelial apparatus has such an extensive literature that as far as possible we shall concentrate here on the lymphocytic element.

Lymphocyte Production

So far as we now know, the continuous production of lymphocytes is the main function of lymphoid tissue, a function so important that Chapter 6 is devoted to its especial consideration.

Metabolism and Transport of Fat and of Protein

Fat Content of Lymph

Since Asellius (1627) first described the lacteals full of chyle, a great deal of attention has been devoted to the part played by the lymphatic apparatus in the absorption of foodstuffs. William Hunter (1784) believed that the lymphatic vessels were the sole channels of absorption. The presence of fat particles in chyle aroused much speculation, and it was generally thought that these chyle "molecules" aggregated to form lymph corpuscles, a view which Brucke (1854) disproved by showing that lymphocytes might be found in large numbers in chyle-free lymph. Fat particles, showing active Brownian movement, can readily be seen under the microscope. Gage and Fish (1924–1925) found that these "chylomicrons" had an average diameter of 0·5 to 1·0 μ, and devised a technique for counting them after they had entered the blood. Chylomicrons appear in the blood a half to one and a half hours after a fatty meal, and

disappear in six to ten hours. The evidence indicates that the fats thus absorbed in particulate form via the chyle are the long-chain triglycerides (see Bloom *et al.*, 1950, 1951; Bergstrom *et al.*, 1950; Frazer, 1952); and also cholesterol (Chaikoff *et al.*, 1952). The fat content of lymph raises a number of problems which are discussed in Chapter 3.

(1) *In lumen of intestine.* Bunting and Huston (1921) believed that large numbers of lymphocytes are constantly passing through the intestinal mucosa and entering the lumen of the intestine Loeper and Marchal (1922) found that after the ingestion of bouillon as many as 1880 leucocytes (80–90 per cent polynuclears) per c.mm. were present in the gastric contents. During conditions of hypochlorhydria these became partly replaced by lymphocytes. Ohno (1930) thought that lymphocytes in the lumen of the intestine were powerful activators of the digestive enzymes, and so assisted in the digestion of protein, starch, and fat by the pancreatic juice. In this connection, we may recall that Bergel (1921) was of the opinion that lymphocytes contained lipase, a view from which Aschoff and Kamiya (1922) and others (cf Barnes, 1940), however, dissented

(2) *In wall of intestine and in lymphoid tissue* Hofmeister (1885) noted a close connection between digestion and the size of the lymphoid nodules in the intestine After feeding, the nodules became enlarged and projected well beyond the surface of the mucosa; at the same time, microscopic examination revealed the presence of large numbers of mitoses During starvation, on the other hand, the nodules underwent shrinkage so that their surfaces became flush with the mucosa, instead of projecting beyond it Zawarykin (1883) and Schäfer (1885) believed that the leucocytes played an important part in the transfer of fat to the lacteals, but they made no accurate differentiation of the white cells The differential staining of white cells had been described by Ehrlich in 1879, but some time elapsed before the discovery was widely applied Heidenhain (1888) confirmed the observations of Hofmeister on the size of the lymphoid nodules, and on their changes during feeding or starvation, but ascribed a minor rôle to the leucocytes in fat transport

Early workers used, as their chief criterion of the ability of cells to take up fat particles, the capacity of the cells to stain with osmic acid, though Heidenhain thought this was unreliable Clearly, the most satisfactory procedure would be to demonstrate the ingestion of fat by living cells Clark and Clark (1917) injected minute amounts of fat (olive oil, oleic acid, cream and egg yolk) into the tails of tadpoles, and observed leucocytes migrating towards the fat and taking up some of the fat particles They did not, in these experiments, distinguish between the various types of leucocytes. In later experiments (1930) they noted that only the macrophages and monocytes were concerned in this process,

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Early workers used, as their chief criterion of the ability of cells to take up fat particles, the capacity of the cells to stain with osmic acid, though Heidenhain thought this was unreliable Clearly, the most satisfactory procedure would be to demonstrate the ingestion of fat by living cells. Clark and Clark (1917) injected minute amounts of fat (olive oil, oleic acid, cream and egg yolk) into the tails of tadpoles, and observed leucocytes migrating towards the fat and taking up some of the fat particles They did not, in these experiments, distinguish between the various types of leucocytes In later experiments (1930) they noted that only the macrophages and monocytes were concerned in this process,

whereas the lymphocytes and granulocytes were quite inert. On the other hand de Winiwarter (1930), working with mice, found that it was only protein feeding that resulted in the accumulation of macrophages —and eosinophils—in large numbers in the mucosa of the intestine. Weill (1919–1920), re-investigating the free cells in the mucous and submucous coats of the intestine, and Leach (1938), studying the rôle of leucocytes in fat absorption, gave critical reviews of the literature. Leach concluded: "There is no evidence that any type of leucocyte plays an important part in the transference of fat from the epithelium to the lacteals"—a view with which we are in agreement. Bloom and Wislocki (1950), re-investigating the distribution of lipids in human blood and bone marrow cells, were unable to find either sudanophilic lipid or phospholipid in the lymphocyte, which was in fact the only white cell to be completely devoid of either.

There has also been considerable difference of opinion as to the relation between fat and lymph nodes Attention was first directed to the mesenteric nodes which appeared milky because of the chyle flowing through them. Holthusen (1910) thought that all the lymph nodes in the body, not only the mesenteric, became filled with fat during digestion Poulain (1902) and Stheeman (1910) believed that there was a reciprocal relationship between the mesenteric and all other lymph nodes. During digestion, the mesenteric nodes became loaded with fat which was being absorbed from the intestine; whereas during starvation, the peripheral nodes became loaded with fat which was being mobilized from the fat depots. Dabelow (1930–1931) was unable to confirm this, even though he tried to stimulate the mobilization of fat by administering thyroxin. Settles (1920–1921) found that feeding animals for several weeks with food rich in fat and protein induced hypertrophy of all the lymphoid tissues, especially the tonsils, lymph nodes and intestinal follicles. Kuczynski (1922) thought that protein was most effective in stimulating the overgrowth of lymphoid tissue. Lefholz (1923–1924), following up the experiments of Settles, fed 3 cats for a period of 16 weeks with high calorie diets in which the extra calories were provided by adding sugar, fat, or proteins, and 2 cats with a diet normal in calorie value, but containing a high percentage of fat or sugar. Peyer's patches and the tonsils were definitely enlarged in the animals which had been given a high calorie diet, but the enlargement was most marked where the extra calories were in the form of fat. "The conclusion, then, seems unavoidable, that the amount of the lymphoid tissue located in the bucco-pharyngeal cavity, and in the intestines, is regulated both by the calorie content, and also specifically by the fat content, of the diet. A high calorie diet, in which the excess calories are provided in the form of sugar or protein, will cause a marked increase in the size of this tissue, while the increase

will be decidedly greater if the excess calories are provided in the form of fat"

Dabelow (1930–1931) made a careful microscopic study of the changes in the mesenteric lymph nodes in the rat, mouse, guinea-pig, and cat during fat absorption, and concluded that there is a definite cycle of changes during digestion. The reticulum cells, most markedly those of the sinuses, become rich in fat during the passage of chyle, and large numbers of these cells, laden with fat, become detached from their network as free macrophages, while the reticulum cells which remain in situ also become swollen up and rounded as they store fat. At the same time, the reticulum cells increase in number, and according to Hansen (1944), in the rat they may with age fuse and form typical giant cells.

The lymphocytes show no sign whatever of being concerned with fat storage, but they undergo cyclical changes nevertheless. After food has been withheld for a brief period, there is diminished multiplication of cells in the germ centres. After prolonged hunger—particularly in the mouse, rarely in the guinea-pig—the germ centres may disappear completely. In the medullary cords and sinuses also, the number of lymphocytes becomes less during starvation. After a hunger period, sudden feeding with foods rich in fat (milk and cream) almost washes away the cells remaining in the sinuses, so that there is left a fine-fibred reticulum with very few lymphocytes Half to one hour after the resumption of feeding following a normal hunger period, however, active multiplication both of macrophages and of lymphoid elements sets in. It is a moot point to what extent hunger in its milder forms can be regarded as a "stress" reaction.

When the passage of chyle is complete, the fat content of the mesenteric nodes diminishes, though it never completely disappears. After prolonged hunger, paradoxically enough, the sinuses and medullary cords again become rich in fats—as rich as during a period of intensive feeding. The fat content of the mesenteric node is, therefore, not a satisfactory criterion for differentiating between hunger and digestion.

The appearance of increased quantities of fat in lymph during starvation was noted by Heidenhain in 1888, and confirmed more recently by Rony, Mortimer and Ivy (1932) These latter investigators found that the thoracic duct lymph of fasting dogs contained a considerable amount of fat, in greater concentration than in the blood. Two experiments also support Dabelow's finding that during digestion the only lymph which contains fat in any amount is that passing through the mesenteric nodes. In these experiments in which 2 dogs were fed with cream and eggs, it was found that in one dog thoracic duct lymph contained 1,580 mg. of fat per 100 ml of lymph, whereas the cervical lymph had only 200 mg.; in

the second dog, the corresponding figures were 4,500 and 210 mg. respectively.

Lymphoid Tissue during Malnutrition and Starvation

There is a very extensive literature on changes in lymphoid tissue during malnutrition and starvation. This has been carefully reviewed by Jackson (1925) for the lymph nodes, thymus, and alimentary canal, and later by Andreasen (1943). The lymphoid tissues appear to be more sensitive than the rest of the body to shortage of food, the thymus being outstanding in this respect and having been designated on that account, " a barometer of nutrition ". The " accidental involution " of the thymus from malnutrition or other causes was investigated by Hammar (1905; 1906). (See also Hammar's review of the problem, 1921.)

Jackson (1925) summarizes the histological findings as follows: " Microscopically, the lymphatic glands during inanition usually show a very characteristic atrophy of the lymphoid tissue, even in cases where *a decrease in the size of the gland as a whole may be offset by a* distension of the blood vessels and lymph sinuses. In general, there is a marked diminution in the number of lymphocytes (by emigration), which renders the less affected stroma (reticulum) and trabeculae very prominent. The lymphoid nodules and cords are reduced in size, and mitoses are decreased in number or absent. Numerous phagocytic cells are found, often containing pigment derived from excessive destruction of erythrocytes (especially in regions of haemorrhage in scurvy). An increased number of phagocytes and plasma cells has been noted during hibernation. Retterer's claim that lymphatic glands may be transformed into haemolymph glands by inanition lacks confirmation Secondary infections may occasion inflammation, however, and occasionally even suppuration of the lymphatic glands, especially in scurvy.

" Cirrhosis of the lymph glands has been noted in pellagra. The lymphoid tissue appears especially sensitive to a dietary deficiency of fat, while in rickets a general lymphoid hyperplasia appears characteristic. A deficiency in vitamins (especially of vitamin B) tends to cause a general atrophy of the lymphoid tissue, associated with lymphopenia in the circulating blood. During chronic thirst, the changes in the lymph nodes resemble those typical for inanition in general, with hyperaemia and lymphoid atrophy

" Although there are numerous variations, the changes in the structure of the lymphatic glands during inanition in general resemble those found in the other lymphoid organs, including the bursa of Fabricius (' cloacal thymus ' *in birds*), *bone marrow . . . and the thymus*, spleen and intestinal lymphoid structures. . . .

" Upon adequate refeeding after inanition, the lymphatic glands in

general recuperate promptly, showing rapid increase of weight, associated with active mitosis and recovery of normal structure in the lymphoid tissue." See also Dustin (1923), Undritz (1924) and Andreasen (1943)

Reactions to Foreign Proteins

In recent years, in connection with the problem of allergy, considerable attention has been devoted to the question of the absorption of undigested protein and to the resulting effects Hamburger and von Reuss (1906) observed the development of leucopenia after the intravenous injection of protein Moss and Brown (1911) gave intravenous injections of protein to non-sensitized and to sensitized rabbits, but with no very definite results. Wiseman (1931*a*) was the first to analyse in detail the cellular response, and to describe clearly the induction of lymphocytosis and of lymphoid hyperplasia following the parenteral administration of protein. It is conceivable, as a result of this work, that many of the lymphoid reactions to bacterial infections may be essentially a response to foreign (bacterial) protein The work of Ehrich (1929*b*), which has already been mentioned, showed that the intravenous injection of killed staphylococci produced a transient lymphocytosis Even more striking, in this connection, are the experiments of Tuta (1937) on *Haemophilus pertussis*

Wiseman's work was performed in rabbits The proteins used were chick embryo extract, egg albumin, and horse serum. The spleen was removed from one rabbit before commencing the injections, in order to see whether the lymphoid reaction would thereby undergo any modification Injections were usually made daily, 6 days per week In most of the animals biopsies were performed before starting the injections and one or more normal lymph nodes were removed for examination. In two experiments the protein injections were subcutaneous, in the others intravenous. Table 39 indicates the effect of these protein injections on the blood lymphocytes and on the spleen. The one splenectomy experiment suggests that removal of the spleen does not modify in any way the general lymphoid response to foreign protein. All 3 proteins injected by Wiseman elicited a lymphoid response, as indicated by lymphocytosis and splenic hyperplasia Intravenous administration of protein seemed to be more effective than subcutaneous, and egg albumin was apparently by far the most potent stimulus to the lymphoid tissues, though, as Wiseman observes, a larger series of experiments would be necessary to decide the point Egg albumin, it may be noted, was the protein of lowest molecular weight in the series (molecular weight = 43,800).

On comparing at the biopsies the lymph nodes removed before the protein injections had begun with those after the injections had been completed, marked hyperplasia was evident in the latter group The

TABLE 39

Changes in blood lymphocytes and weights of spleens after parenteral protein injections §

Rabbit	Material injected	Route	Pre-injection period				Injection period				
			No of injections	*Average no lymphocytes per cmm of blood*	*No of counts made*	*Weeks observed*	*Average no lymphocytes per cm m of blood*	*No. of counts made*	*Weeks observed*	*Percentage increase in lymphocytes*	*Approximate percentage increase in weight of spleen at autopsy*
R1160	Embryonic extract	Intravenous	45	3,179	13	5	5,599	44	11	76	300
R1157	,, ,,	,,	19	2,477	21	7	3,911	19	6	58	600
R1111	,, ,,	Intraperitoneal	13	3,723	10	4	4,572	18	3	23	400
R1158	,, ,,	Subcutaneous	24	3,507	18	7	5,151	24	5	46	None
R1163	Egg albumen *	Intravenous	25	3,208	20	8	6,500	27	5	102	250
R1183	,, ,,	,,	37	2,436	10	13	4,705	14	5	97	200
R1264	,, ,,	Subcutaneous	13	2,356	11	5	3,555	13	5	59	400
R1180	Horse serum	Intravenous	37	3,516	7	13	4,508	14	5	28	300
R1185†	Egg albumen	,,	37	2,504	11	13	5,983	13	5	139	—
R1187†	None	—	—	3,067	10	4	2,572	16	6	16‡	—
R1186	Normal salt	Intravenous	37	1,793	10	13	2,035	15	5	13	None

* 6 mg albumen per injection in this animal † Splenectomized. ‡ Decrease § From Wiseman (1931a, p. 501).

cortical nodules were enlarged and there was a marked increase in the number of mitotic figures in the germinal centres. "The medullary cords were, in some cases, almost obliterated by the encroachment and invasion of lymphoid tissue. Many of the cortical nodules were confluent, resulting, in some cases, in the node assuming the appearance of diffuse hyperplastic lymphoid tissue, as contrasted with the ordinary distinct corticomedullary architecture of the original normal node from the same animal." It is very interesting to note, however, that there was no marked difference in weight between the normal and the hyperactive nodes The weight of the node is evidently not a reliable guide to its lymphocytopoietic activity. There was no sign of proliferation of thymic tissue and Wiseman interpreted this as casting further doubt on the identity of the small thymic cells with the true blood lymphocytes; in the light of more recent work (p. 273) the absence of any thymic response to foreign protein may well be due to the lack of plasma cells. It is not clear whether the response of the lymph node is to protein reaching it via the blood or the lymph—or possibly both Latta (1951) found that "Iodinated serum albumen . was concentrated in the medullary portion of the lymph nodes quite out of proportion to the blood content". It will be recalled that it is in the medullary cords that there occurs a marked formation of plasma cells, which we have already noted as the most likely source of antibody.

A significant feature of the lymphocytosis in Wiseman's experiments was that it was associated with qualitative changes in the lymphocytes In supravital preparations there was a great increase in the number of intermediate and large lymphocytes, with basophilic cytoplasm containing large numbers of mitochondria Elsewhere Wiseman (1931*b*) had given his reason for believing that basophilia tends to be associated with young and immature cells Hence, the intermediate and large lymphocytes which appeared in large numbers in response to foreign protein were also, in his opinion, young and immature.

It should perhaps be emphasized that at the time Wiseman performed his experiments little was known about the true significance of cytoplasmic basophilia and its relation to new protein formation, most frequently in connection with cell growth, though in the case of a cell such as the plasma cell in relation to gamma globulin production (Fagraeus, 1948; Keuning and van der Slikke, 1950)

But whatever may be the rôle of the different classes of lymphocytes, there is no doubt about the stimulating action of foreign proteins on the lymphoid tissues as a whole, and Wiseman's work constituted a fundamental addition to our knowledge Oliver and Katzman (1938) reported the production of an almost leukaemic (lymphatic) condition in mice following injections of sodium caseinate. The experiments of Kolouch

(1939) showed that in rabbits the subdermal injection of egg albumin resulted in a local accumulation of small lymphocytes, present in considerable numbers at 8 hours after the injection. By the fourteenth hour many of them were enlarging and becoming transformed into macrophages.

It is fitting at this point to ask ourselves to what extent the lymphoid tissue may normally be called upon to deal with foreign protein; or in other words, to what extent is foreign protein normally absorbed into the blood without being first broken down by digestive enzymes. Since the first observations of Uhlenhuth (1900) and of Ascoli and Vigano (1903) the problem has been investigated by many workers and has been extended to cover several kinds of protein. (For a review of the literature, see Ratner and Gruehl, 1934.) Much of the earlier work was done with egg albumin, though Hektoen, Kanai and Dragstedt (1925) used thyroglobulin, Walzer (1927), fish protein; and Ratner and Gruehl (1934), milk. Alexander, Shirley and Allen (1936) confirmed what had been a matter of dispute among previous workers—that undigested egg albumin administered to dogs by stomach tube could be detected in thoracic duct lymph. Yoffey, Sullivan and Drinker (1938) showed that after nasal instillation egg albumin could be identified in cervical lymph.

Hartley (1942) showed that in guinea-pigs the oral administration of crystalline egg albumin frequently gave rise to the formation of circulating antibodies. The results of Walzer (1927) and of Brunner and Walzer (1928) are particularly interesting. Using the Prausnitz-Kustner method of passive transfer of reagins, they found that in sixty-one patients, out of sixty-five, there was absorption into the blood of undigested fish protein. More recently Abdou, Reinhardt and Tarver (1952), using protein labelled with C_{14}, have shown that in rats, protein after oral administration could frequently be found in small amounts in thoracic duct lymph. Apparently one must regard as a normal process the absorption of small amounts of undigested protein, and it is possible that to deal with this is part of the function of normal lymphoid tissue.

Vitamin Storage and Vitamin Deficiency

*A discussion of the vitamin content of lymphoid tissue almost inevit*ably includes consideration also of the effects on lymphoid tissue of vitamin shortage. With regard to the latter, it is difficult at times to be certain that one is dealing with the uncomplicated effects of vitamin deficiency, since the picture is often confused by secondary changes such as infection.

Vitamin A

Turner and Loew (1930–1931) concluded that " leukocytosis with an increase in the per cent of polymorphonuclear leucocytes and a decrease

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underwent marked atrophy in A-hypovitaminosis, and hypertrophied when an excess of vitamin A was administered. Money, Fager, Lucas and Rawson (1952) reported that increased intake of vitamin A caused an increase in weight of thymus and lymph nodes, and also considerably lessened the thymic atrophy induced by Reichstein's compound S The lymph nodes hypertrophied after compound L, and it might have been thought that compound L and vitamin A in conjunction would cause an even greater hypertrophy than either alone. Somewhat unexpectedly, however, if large doses of vitamin A were given with the compound L, the hypertrophy of the lymph nodes was less than when the vitamin A was given in small doses. Money and his co-workers (loc cit) incline to the view that vitamin A does not act directly on lymphoid tissue, but through the mediation of the thyroid gland.

Vitamin B

Most of the observations on the relation between vitamin B and the lymphoid tissues were made before the B complex began to be resolved into its component elements McCarrison (1921) found that in pigeons, polyneuritic from deficient vitamin B, there was atrophy of the intestinal lymphoid tissue. In monkeys with vitamin B deficiency, the lymphoid elements in the intestinal mucosa were greatly diminished, though there was swelling of lymphoid nodules in the colon, and also enlargement of the draining mesenteric nodes, suggesting infection. Uotila and Simola (1938) could detect only slight atrophy of lymphoid tissue in vitamin B deficiency. Cramer, Drew and Mottram (1921*a* and *b*) concluded that there was a specific relationship between vitamin B and the lymphoid tissue: " Absence of the water-soluble B-vitamin from the diet leads in

not lead to an atrophy of the lymphoid tissues and there is no lymphopaenia The lymphopaenia established by withholding the water-soluble B-vitamin is rapidly abolished by the administration of the water-soluble B-vitamin, and *pari passu* with it, the concomitant marasmus "

The observations of Cramer, Drew and Mottram raise the question whether lymphoid tissue normally contains vitamin B Hammar (1937), on the basis of somewhat indirect evidence, concluded at first that such was the case He found that the growth of tadpoles could be stimulated by extracts of thymus, lymph nodes, or muscle, as well as vitamin B

a second group with relatively high concentration. The heart and kidneys are in a third lower group, distinctly above the muscle tissues and blood" Von Euler and Klussman (1933) reported a high concentration of vitamin C in the thymus. This was confirmed by Yavorsky, Almaden and King (1934), who found that in infants up to the age of 12 months the thymus contained 0·311 mg. of ascorbic acid per g. (mean of 20 cases); whereas from 1 to 10 years the figure sank to 0·190 mg. per g. (mean of 11 cases). Only 2 specimens of thymus could be obtained from individuals between 46 and 77 years of age, with a vitamin C content of 0·046 mg. per g. De Ludany and Zselyonka (1937), working with dogs, found that lymph nodes contained 0·310 mg. ascorbic acid per g. of tissue (mean of 15 estimations), while the tonsils contained 0·287 mg per g. (mean of 7). Zimmet and Dubois-Ferrière (1937*b*) obtained for the tonsil a comparable figure—0·23 mg. of ascorbic acid per g. They also noted (1937*a*) that saliva normally contained some vitamin C, which after tonsillectomy fell to about half its preoperative level. Zimmet and Dubois-Ferrière (1937*c*) found that the normal appendix contained ascorbic acid—0·17 mg. per g. —whereas in the inflamed appendix there was present only 0·10 mg per g Hammar (1938*b*), in two calves, found that the thymus had a vitamin C content of 1·65 and 2·05 mg. per g, the corresponding figure for lymph nodes being 0·95 and 1·65 mg., whereas muscle only had 0·12 mg. per g. Fujita and Ebihara (1937) also found vitamin C in the thymus.

For the microscopic detection of vitamin C, Giroud and Leblond (1935) used silver nitrate and demonstrated vitamin C in various organs, but not in the thymus. Subsequently Zimmet and Dubois-Ferrière (1937*c*) demonstrated vitamin C in sections of the appendix, and Hammar (1938*b*), in thymus In both these instances it was concluded that the ascorbic acid was present in the reticulo-endothelial cells, but not in the lymphocytes Hammar went further than this, and argued that since his tadpole experiments (1937) had proved to his satisfaction that thymus and lymph nodes contain vitamin B, and since it had further been shown that vitamin C was located in the reticulo-endothelial cells, therefore the vitamin B must be present in the lymphocytes. It is difficult to reconcile this conclusion with the observations of Stephens and Hawley (1936), who showed that lymphocytes from the blood of leukaemic patients always contained vitamin C, sometimes in considerable quantities. A similar result was obtained by Butler and Cushman (1940), who investigated the vitamin C content in 9 cases of leukaemia (7 of them being lymphatic). Kellie and Zilva (1938) found vitamin C in guinea-pig leucocytes (blood) of which the majority are lymphocytes.

What is the significance of the relatively high vitamin C content of lymphoid tissue? Hammar (1937) was of the opinion that the vitamin C content of the thymus might be associated with a detoxicating and immun-

izing function—a function which he had previously suggested on other grounds. Bessey and King (1933) attribute a more general biological significance to the presence of vitamin C. " The finding of as much vitamin C in the corpus luteum as in the adrenal cortex was of particular interest in corroborating the general physiological relationship of the vitamin to (*a*) a high respiratory rate, (*b*) complex lipids, and (*c*) rapidly growing tissue. It minimizes the probability of a direct specific relation to the adrenal cortex, which has been considered frequently because of

... It appears probable that the vitamin

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any particular tissue. . . . In younger animals the vitamin C content of the tissues tends to be higher than found for older animals." If—as we believe—this interpretation is correct, it is in close accord with the conclusion previously drawn from the effects of irradiation; namely, that the lymphoid tissues are young and actively growing.

Wolbach and Maddock (1952) found that in guinea-pigs quite a severe degree of scorbutus could be induced without any obvious effect upon lymphoid tissue. It has been suggested (Schnetz, 1938) that vitamin C has a regulatory influence on the white cell count in the circulating blood, tending to depress a high count or raise a low one. The results are not very convincing and Gingold (1937) from his observations concludes that ascorbic acid is absolutely without effect on the blood leucocytes.

However, even if this is the case, it may nevertheless be that ascorbic acid does influence some other factor which in turn acts on the blood cells. Thus May *et al.* (1951) report the production in monkeys of an experimental megaloblastic anaemia by feeding milk diets deficient in

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upsetting the metabolism of pteroylglutamic acid.

Vitamin D

Reference will be made elsewhere to the frequent development in rickets of lymphoid nodules in the bone marrow. This raises the question of the influence of vitamins A and D on the lymphoid tissues. Vitamin A has already been considered; as to D, Nitschke (1932) was of the opinion that it has an inhibitory effect on lymphoid tissue. This, if it were true, would explain the overgrowth of lymphoid tissue in rickets. Ackermann (1937–1938), however, concluded that precisely the opposite was the case, having found that feeding hedgehogs with vitamin D produced marked proliferation of lymphoid tissue and increased formation of small lymphocytes

Elaboration of Internal Secretion

The evidence bearing on the possibility that the lymphoid tissues possess an endocrine function, while not inconsiderable, is nevertheless inconclusive (see Marfori *et al.*, 1934), and we shall not discuss it here.

Destruction of Red Cells. Haemolymph Nodes

Inasmuch as many of the lymphoid tissues contain reticulo-endothelial cells, they may be regarded as playing a minor part in the destruction of erythrocytes (see review by Jaffe, 1938), a function discharged more especially in the so-called haemolymph nodes (Warthin, 1901–1902; Weller, 1938) *Haemolymph nodes are located mainly in front of* the lumbar and cervical vertebrae, though in some animals subcutaneous nodes have been described. In man, they tend to be much smaller than ordinary lymph nodes.

Haemolymph nodes raise a number of interesting problems, and—like other lymphoid tissues—have been the subject of much controversy. Their lymphoid elements appear to be indistinguishable from those in other lymphoid tissues and their biological reactions are the same; though if, as is sometimes stated, communications with lymphatic pathways do not exist, lymphocytes could only reach the blood stream by migrating through the wall of blood vessels.

In general, it may be said that haemolymph nodes have been interpreted in two essentially different ways, well typified in two recent papers One view is that they are normal lymph nodes in which an unusually large number of erythrocytes enter the lymph sinuses, but in which the normal process of lymphocytopoiesis continues in varying degrees. The other view is that they can give rise to cells other than lymphocytes.

The former view has been restated by Andreasen and Gottlieb (1946), who find haemolymph nodes appearing in the lumbar region of rats at *about the age of one month. These start as ordinary lymph nodes*, but soon an increasing number of red cells begins to accumulate in the sinuses and many of them are ingested by macrophages, which because of their erythrocyte content are frequently referred to as erythrophages.

In this type of node the question obviously arises as to the source of the erythrocytes. It will be recalled that small numbers of red cells normally escape through the walls of the blood capillaries into the pericapillary tissues and are then removed by entering lymphatic capillaries, so that they can be found in peripheral lymph (Yoffey and Drinker, 1939). These cells might then be held in the lymph nodes (Drinker, Field and Ward, 1934). It follows that anything which damages the endothelium of the blood capillaries will greatly increase the normal extra-

vasation of red cells into the tissues and will finally, when these erythrocytes are removed via the lymphatics, lead to a typical haemolymph transformation of the regional lymph nodes. This mechanism is undoubtedly responsible for the development of haemolymph nodes after the administration of 1 : 2 : 5 : 6-dibenzanthracene (Lasnitski and Woodhouse, 1944), or after irradiation. Reference has already been made to the appearance of large numbers of red cells in lymph following X-irradiation, as in the experiments of Ross, Furth and Bigelow (1952)

However, in the case of the lumbar haemolymph nodes of the rat, Andreasen and Gottlieb postulate another mechanism for the accumulation of red cells in the lymph sinuses, and they attribute it to a reflux of blood from the renal vein into an efferent lymphatic which drains into the vein. Following nephrectomy, which effectively prevents this reflux, the haemolymph node becomes a normal lymph node. Selye and Schenker (1939) had also demonstrated this reversion of a haemolymph node, after either homolateral nephrectomy, or nephrectomy combined with adrenalectomy

The second interpretation has been put forward by Winqvist (1954), who reports the occurrence of active myelopoiesis in the haemolymph nodes of cattle This type of haemolymph node could well be regarded as an extension into adult life of what is, after all, a normal occurrence during embryonic life, or if commencing *de novo* it could also be regarded as an example of myeloid metaplasia

Selye and Foglia (1939) describe a generalized formation of pseudo-haemolymph nodes in rats during the "alarm" reaction.

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CHAPTER 6

CELL CONTENT OF LYMPH

General Significance

Lymph normally contains a few red cells and a varying number of white cells, depending on the region from which the lymph is obtained The only white cell ever present normally in large numbers is the lymphocyte. Lymphocytes are being continually produced in the lymphoid tissues, many of them entering the lymph stream and passing through the thoracic and right lymphatic ducts before they reach the blood It is thus possible by tapping these ducts to collect and study quantitatively lymphocytes entering the blood via the lymph stream Indeed, the lymph-borne lymphocytes are the only blood cells whose output we are able to measure directly. The remaining white blood cells—for the most part granulocytes—and the red cells are entering the blood diffusely from the marrow of the various bones, or from organs such as the spleen, and their numbers can only be assessed indirectly.

Peripheral, Intermediate, and Central Lymph

The number of lymphocytes in lymph varies depending on the kind of lymph in which they are found. In this connection we distinguish between three varieties of lymph *peripheral* lymph, which has not yet passed through a node or come into contact with other lymphoid masses; *intermediate* lymph, which has already been in contact with some lymphoid tissue but has yet to pass through more; and *central* lymph, which is on its way to the blood without further interruption Lymph from the hind limb of an animal below the knee, or from the fore limb below the elbow, is peripheral. Lymph from a mesenteric vessel may be peripheral or intermediate if draining a segment of intestine devoid of organized lymphoid tissue through which it must flow [illegible]

the thoracic duct Thoracic duct lymph is central. Cervical lymph as obtained in most experiments is usually central, since the cervical duct is cannulated low down in the neck where its lymph has no further nodes to traverse. As a rule, peripheral lymph contains few white cells; intermediate lymph, more; and central lymph, the greatest number.

Peripheral Lymph

Lymph obtained from the fore or hind limbs of anaesthetized animals seldom flows spontaneously. In the rare instances in which we have observed a spontaneous flow, it has been associated with an abnormally high cell content and high protein, and we have regarded these cases as pathological. As a rule, lymph from the limbs of quiescent animals can only be obtained by massage, a manoeuvre which does not appear to inflict obvious damage either to the blood or lymph capillaries (Yoffey and Drinker, 1939*a*). It has been our experience that red cells are never found in large numbers in the lymph obtained by massage, nor is there, during the experimental period, a progressive increase in the white cell content. If the capillaries were damaged one might also expect an increased leakage of protein through their walls, but in determinations carried out over several hours on lymph obtained by massage, we have never found a marked change in the protein content.

In the actively moving dog lymph from the limbs flows relatively freely. Haynes and Field in 1931 cannulated leg lymphatics of dogs under local anaesthesia. In a typical experiment, the first lymph collected showed an occasional red cell in a film preparation. After 11 minutes' walking, with lymph flowing freely, the lymph contained 2,200 red cells and 240 white cells per c mm. Thirteen minutes' running brought this up to 3,600 red cells, with 220 white cells ; and after 14 minutes more the red cells were 13,600 and the white cells 300. This type of result was obtained on a number of occasions; and there can be no doubt that, with

that light pressure may be sufficient to cause cells to pass from blood to lymph capillaries. The fact that lymph obtained by massage does not even show an increase in cell content, such as occurs in walking, indicates that the lymphatics are not easily damaged by the usual experimental manipulations.

Table 40 presents data on the lymphocyte and the total white cell count of peripheral lymph. The making of accurate differential counts in lymph presents a distinct problem. Rous (1908*c*) first pointed out the difficulty of making satisfactory films of lymph, and attributed it to the lack of " body " as compared with blood, due presumably to the lower protein content The protein content of lymph from the extremities is usually even lower than that of thoracic duct lymph with which Rous was working, and hence the difficulty becomes accentuated.

The differential counts in Table 40 were made in the counting chamber of the haemocytometer, where one can readily distinguish lymphocytes and polymorphonuclear cells, but not so readily between lymphocytes and

TABLE 40

Total white cell and lymphocyte count per c.mm in peripheral lymph, and lymphocyte count in thoracic duct lymph and blood

No. of experiment	Peripheral lymph					Blood	Thoracic duct lymph
	Total white cells						
	No. of counts	Highest	Lowest	Mean	Lymphocytes	Lymphocytes	Lymphocytes
				Dogs			
1	12	625	50	270	170	1,100	—
2	3	225	25	150	120	—	—
3	12	1,625	75	670	380	500	—
4	1	175	175	175	150	1,200	—
5	9	350	0	140	70	—	—
6	8	1,950	225	620	400	1,700	—
7	3	475	50	170	85	950	—
8	9	275	0	130	50	370	—
9	5	2,000	800	1,190	635	3,750	—
10	7	975	150	560	400	1,900	—
11	7	2,500	475	1,100	510	1,600	12,600
12	9	225	25	90	40	900	12,050
13	2	175	100	140	75	2,000	—
14*	7	3,925	750	2,300	580A 1,450B	300	2,300
15	10	1,800	175	810	690	800	4,400
16	6	700	100	350	140	1,400	—
Mean				550	280	1,320	7,800
				Cats			
17	3	825	300	500	460	—	—
18	6	1,375	150	520	360	5,300	—
19	3	625	250	425	320	3,040	13,900
20	3	625	525	580	470	1,100	5,100
21	3	750	50	460	370	2,100	17,100
22	3	375	225	310	240	7,300	—
23	3	600	350	450	360	3,400	—
Mean				430	370	3,700	12,000

* Two mesenteric vessels were cannulated, draining A, the upper end of the jejunum, B, the lower end of the ileum B probably drains a Peyer's patch, and its lymph cannot therefore be regarded as peripheral In calculating the mean lymphocyte count in peripheral lymph, B has been omitted

From Yoffey and Drinker (1939*a*)

other mononuclears. Supravital staining showed, in the few counts done, that peripheral lymph contains an unusually high percentage of monocytes —reckoned as lymphocytes in Table 40.

Allen (1945) made counts on the cell content of peripheral lymph (diaphragmatic) in rabbits and also found a low lymphocyte content. Allen further concluded that peritoneal fluid, which he regarded as identical with tissue fluid, contained approximately five times as many leucocytes as peripheral lymph, a fact which he explained by assuming that lymphatic endothelium constantly " discriminates " against the passage of lymphocytes, and that the main function of these cells is therefore discharged in the tissue spaces. This question is further discussed on page 357.

Osogoe (1943) gave an interstitial injection of autogenous lymphocytes and concluded that they returned to the blood by passing through the walls of the blood capillaries, not by entering the lymphatics The cell content of peripheral lymph was attributed to the presence of small masses of lymphopoietic tissue in walls of lymphatic vessels, following some observations of Hitachi. While it is impossible to disprove the existence of such foci, it is doubtful whether they are of widespread occurrence in mammals, though we have previously (p. 24) referred to their not infrequent occurrence in birds. But if they do occur they would reduce still further the likelihood of a lymphocyte circulation

Intermediate Lymph

Table 41 contains a number of white cell counts made by Baker (1932–1933) on lymph from various parts of the small intestine of cats. On the average lymph draining a Peyer's patch contained approximately ten times as many lymphocytes as lymph from an area devoid of lymphoid tissue. Baker's table also gives data for the same lymph after passing through the mesenteric node—i e. central lymph—and this contained six times as many cells as intermediate lymph from a Peyer's patch. The vast majority of the cells were said to be lymphocytes, though no counts are given.

Baker (1932–1933) also paid attention to the lymphocyte content of intestinal lymph during digestion. " The impression was gained . . . that the cellular content of chyle was inversely proportional to the fat content. This finding lends no support to the commonly expressed view that leucocytes, and especially lymphocytes, are probably important agents in fat transportation. It is clear that they cannot be important in carrying out such a function in chyle, for every sample of chyle, especially when not coming from a Peyer's patch, showed an insignificant number of cells in relation to the large quantity of fat. Whether leucocytes carry fat particles from the intestinal epithelium through the tissue of the villus and into

the central lacteal . . . was a problem not included in the present work."

Central Lymph

Cervical Lymph. Few observations have been made on the cell content of cervical lymph. Table 42 includes counts by Davis and Carlson (1909–1910) and Haynes and Field (1931) in the dog, by Reinhardt and Li (1945) in the rat, and by Reinhardt and Yoffey (1955) in the guinea-pig. In the dog experiments, lymph was obtained by repeated massage of the cervical duct. If, as not infrequently happens, this massage involves the node also, many more cells are set free and the count in the efferent lymph rises, as had previously been shown by Florey (1927) as well as by the other investigators just quoted. This source of error is obviated by the technique described by McCarrell (1939*a*) for obtaining in the dog a steady flow of cervical lymph without massage. In the rat and the guinea-pig cervical lymph usually flows spontaneously; in the cat, and still less in the rabbit, lymph rarely flows spontaneously, but can only be obtained by massage.

In the fully anaesthetized monkey a slight spontaneous flow of cervical lymph is the rule rather than the exception. This is possibly aided by the movements of respiration, which result in a certain amount of massage of the cervical lymphatics by adjacent muscles. Table 43 gives a number of cell counts made on the cervical lymph of monkeys. These counts, which represent eventual additions to the blood lymphocytes are, no doubt, lower than would be found in the cervical lymph of unanaesthetized animals, in whom swallowing and muscular movements would increase lymph flow and cell counts.

Thoracic Duct Lymph. Many estimations of the cell content of thoracic duct lymph have been made. Erythrocytes are always present, though their number varies greatly. Occasionally one must search for some time in order to find a single cell, but usually they are fairly plentiful, and their number can readily be increased by abdominal massage and by passive or active movements of the extremities. Rous (1908*a*) apparently believed that these red cells had entered directly from the blood. Occasionally we have seen a small vein connecting with the thoracic duct close to its termination, but this is exceptional and cannot account for the usual occurrence of red cells in thoracic duct lymph. It must be remembered in this connection that red cells are normally present in peripheral lymph.

Counts of the total number of white cells in thoracic duct lymph are given in Table 44. Differential counts are to be found in practically all the references in this table. It is apparent that the actual number of cells in thoracic duct lymph is a very variable quantity and that most of the cells are lymphocytes. The findings of Rous (1908*a*) for the dog,

TABLE 41

Cellular content of chyle in cats as determined by counting fresh samples

	Chyle from prenodal lacteals							Chyle from postnodal lacteal	
	Draining no Peyer's patch			Draining a Peyer's patch					
Cat no and wt in kg	*Distance above ileo-caecal valve, cm*	*Time in minutes*	*White cells per c mm*	*Distance above ileo-caecal valve, cm.*	*Time in minutes*	*White cells per c mm.*	*Time in minutes*	*White cells per c mm*
I	68	—	933	—	—	—	—	15,175
2 1	55	—	2,500	—	—	—	—	—
kg								
II	65	5	1,430	14	20	5,673	35	19,910
2	32	10	292	25	25	5,170	40	5,673
kg	69	15	110	—	—	—	—	—
	29	30	165	—	—	—	—	—
III	53	10	602	1 5	14	47,200	45	18,771
1 4	18	20	277	—	—	—	50	14,003
kg	29	24	771	—	—	—	—	—
	38	28	1,157	—	—	—	—	—
	68	35	500	—	—	—	—	—
	25	40	1,023	—	—	—	—	—
IV	8	10	175	41 5	19	3,200	50	1,690
2 7	34	15	264	46	24	550	—	—
kg	62	28	120	4·5	39	1,462	—	—
	28	34	340	12	43	300	—	—
	—	—	—	78	48	3,510	—	—
V	50	13	2,000	7	10	2,200	37	315
2	97	20	330	76	17	1,600	—	—
kg.	110	23	286	—	—	—	—	—
	116	27	300	—	—	—	—	—
	93	33	400	—	—	—	—	—
VI	13	5	333	—	—	—	dead	12,222
1	35	10	666	—	—	—	"	5,360
kg.	51	13	248	—	—	—	—	—
	57	17	1,025	—	—	—	—	—
	79	22	2,084	—	—	—	—	—
VII	3	23	1,666	13	12	9,454	17	152,224
1	9	27	1,110	40	31	1,639	43	9,640
kg.	51	34	333	—	—	—	—	—
	54	40	0	—	—	—	—	—
VIII	66	17	1,200	—	—	—	30	143,800
2·2	—	—	—	—	—	—	34	129,800
kg.								
Average			730	—	—	6,829	—	41,199
Correction for free peritoneal cells			31	—	—	31		
Corrected average			699	—	—	6,798	—	41,199

From Baker (1932–1933)

TABLE 42

White cell counts in lymph from vessels other than the thoracic duct

Observer	*Kind of lymph*	*Total white cell count per cubic millimeter*	*Differential*	*Animals used*
Winternitz (1895)	Leg lymph Thigh	403– 1,173	—	Dog
Davis and Carlson (1909–1910)	Cervical lymph	5,000–24,722	Practically 100 per cent small mononuclears	„
Haynes and Field (1931)	Cervical lymph	4,075–27,325 average 12,772	95 per cent lymphocytes 4 per cent large mononuclears	8 dogs
	„ „	2,800–68,600	98 per cent lymphocytes, 2 per cent large mononuclears	7 „
	Kidney lymph	1,200	—	Dog
	Leg lymph at femoral triangle	0–2,500 average 520	—	7 dogs
	Leg lymph at ankle	0–100 average 33	—	3 „
Florey (1927*a*)	Lymph from efferent vessel of mesenteric lymph gland	1,400–36,875	Mostly lymphocytes. A small number of large mononuclear cells	Cat
Menkin and Freund (1929)	Lymph from efferent of axillary lymph node	9,000–55,000	100 per cent lymphocytes	Rabbit
Reinhardt and Li (1945)	Cervical	1,500–17,500 (7,065 1,725)	Almost 100% small and medium lymphocytes	37 rats
Mann and Higgins (1950)	Intestinal (Receptaculum)	16,700–37,000	Practically all lymphocytes. Mainly small	8 rats
„ „	Hepatic	6,200–19,900	„ „	4 rats
Reinhardt and Yoffey (1955)	Cervical	6,520 ± 1,630	Practically all small lymphocytes	6 guinea-pigs

and of Kindwall (1927) for the rabbit, are characteristic, and are given in Tables 45 and 46. (See also Sanders, Florey and Barnes, 1940, cat and rabbit; Reinhardt and Yoffey, 1955, guinea-pig.) Rous (1908c), using dry smears, found in thoracic duct lymph an average content of 0·39 per cent of transitional cells—i e. monocytes. Later workers (Simpson, 1922; Kindwall, 1927; Bloom, 1928a; Yoffey, 1932–1933), employing the supravital staining technique, all seem to be agreed that normal thoracic duct lymph contains practically no monocytes As to eosinophiles, Rous (1908c) found in the dog an occasional high count, though the mean of his series was only 2 6 per cent.

From a review of the literature it appears that in making white cell counts of normal thoracic duct lymph, all the mononuclears seen in the counting chamber of the haemocytometer may safely be reckoned as lymphocytes. The error thus introduced by including among the lymphocytes an occasional monocyte or macrophage is usually negligible. Under

TABLE 43

Lymphocytes and protein in cervical and thoracic duct lymph and protein in blood plasma of the monkey (*Macaca Mulatta*)

No. of animal	*Cervical lymph*		*Thoracic duct lymph*		*Blood plasma protein in mg*
	Lymphocytes per c mm	*Protein, g per cent*	*Lymphocytes per c mm*	*Protein, g per cent*	*per cent*
1	—	—	38,000	3 72	6 25
2	—	—	15,200	3·59	5 48
3	—	—	16,600	—	—
4	—	—	11,800	—	—
5	10,200	3 23	—	—	5 2
6	20,900	2 25	—	—	4 49
7	—	—	—	—	4 62
8	12,350	4 14	—	—	5 92
9	57,500	4 29	—	—	4 96
10	—	3 67	—	—	—
11	9,350	2 74	—	—	—

From Drinker and Yoffey (1940)

conditions of abnormal stimulation, this may not hold good. Thus Simpson (1922) found that after intravenous injections of Niagara blue a fair number of macrophages were to be found in thoracic duct lymph, but no monocytes. Similarly Kiyono (1914) found, after intravenous injection of carmine into rabbits, that 5 to 8 per cent of macrophages appeared in thoracic duct lymph. Curiously enough, plasma cells are not often seen in any numbers in thoracic duct lymph, though it has been claimed that as many as 6 per cent of "plasmoid" cells may be found

in intermediate lymph draining nodes in which there is active antibody formation (Matsamura, Tanaka and Takenaka, 1952)

It should perhaps be emphasized that an appreciable number of large blast-like cells (5 per cent Bierman *et al*, 1953; 3-5 per cent Reinhardt and Yoffey, 1955) may be found in thoracic duct lymph. The nature and fate of these cells is not clear. Because of their large size it may be that they are filtered out of the blood in the pulmonary capillaries. They may sometimes be seen in mitosis in thoracic duct lymph, and it may well be these cells which are capable of producing antibody in tissue culture (Keuning and van der Slikke, 1950; Wesslén, 1952*a*).

TOTAL LYMPHOCYTE OUTPUT OF LYMPHOID TISSUES

In attempting to assess the total lymphocyte output of lymphoid tissues it is necessary to consider the sites of lymphocyte formation and the possible channels through which lymphocytes may enter the blood Lymphocytes may be formed. (1) in the lymph nodes and lymphoid tissue of the alimentary canal, (2) in lymph nodes in other parts of the body—e g limbs, or head and neck, (3) in the spleen, (4) in the thymus; and (5) in the bone marrow. In addition, there may be scattered through the tissues small unencapsulated collections of lymphocytes

Lymph Nodes and Lymphoid Tissue of Alimentary Canal

Many lymph-borne lymphocytes are formed in the lymph nodes and lymphoid tissue of the alimentary canal, and later enter the blood via the thoracic and right lymph ducts It is probable, in fact, that the mesenteric lymphoid tissue is the main source of the blood lymphocytes (Yoffey, 1933 and 1936, Sanders, Florey and Barnes, 1940, Mann and Higgins, 1950) There may however be other channels than the thoracic and right lymph ducts through which these cells can reach the blood, one of them being lymphatico-venous communications in the abdomen and thorax (Job, 1918, Andreasen and Gottlieb, 1947). This does not introduce a fundamental difference; lymphocytes still enter the lymph and are conveyed by it to the blood, but the irregular occurrence and small size of these lymphatico-venous communications make their investigation extremely difficult

Another channel of a different nature, namely the so-called stomata in the endothelium of the postcapillary veins of the lymph nodes, has been suggested by a number of workers (Zimmerman, 1923; Schulze, 1925; Bloom, 1938, Sanders, Florey and Barnes, 1940) It is true that one may occasionally see what appears to be a lymphocyte migrating through a vessel wall, though the direction of the migration is not clear; but

TABLE 44

White cell content of thoracic duct lymph

Observer	*Cells per c mm.*	*Animal and remarks*
Haedicke (1906)	2,000–20,000	Man. Chylus fistula
Ranvier (1875)	4,800– 7,500	Dog
Winternitz (1895)	1,372–22,729	Dog Morphine No food 24 to 48 hours
Biedl and von Decastello (1901)	3,200– 6,480	Dog Chloroform
Goodall and Paton (1905–1906)	5,600–17,000	Dogs after feeding meat and cream Ether Thoracic duct lymph
Rous (1908a) . . .	990–11,160	Dog Morphine and chloroform No food 24 to 48 hours
Davis and Carlson (1909–1910) .	1,000–30,000	Dog Ether.
Chistoni (1909) .	6,400–13,360	Dog. Morphine
Osato (1922) .	1,600–20,500	Dog Morphine and ether
Haynes and Field (1931)	500–12,250	Dog Sodium barbital
Goodall and Paton (1905–1906)	10,000–72,000	Cats after feeding cream. Lymph from receptaculum chyli
Ranvier (1875) . .	11,300	Rabbit
Bunting and Huston (1921)	20,000–50,000	Rabbit. Lymph taken just after death
Kindwall (1927) . . .	20,466–44,960	Rabbit. Sodium barbital
Forgeot (1907)	1,950–61,750	Goat
	4,600–28,875	Cow
Sanders, Florey and Barnes (1940) .	5,900–20,700	4 cats. Starved
Sanders, Florey and Barnes (1940)	6,000–28,300	6 cats Fed
Sanders, Florey and Barnes (1940)	8,500–66,800	1 rabbit. 6 counts at intervals of ½ hr.
Adams, Saunders and Lawrence (1945)	14,150 (mean) 4,300–29,600	21 cats Fed with cream four hours before cannulation
Reinhardt (1946)	22,800 ± 1,960	23 rats. Mean ± standard error of mean
Valentine, Craddock and Lawrence (1948)	6,400–28,800	10 cats Counts taken in each case on lymph collected during first hour after cannulation
Mann and Higgins (1950)	10,900–88,100	22 rats. Lymph collected from cannula in thoracic duct after recovery from anaesthetic
Courtice, Simmonds and Steinbeck (1951)	1,850– 3,800	Man. Case of thoracic duct fistula which discharged for about one month Malignant disease
Bierman, Byron, Kelly, Gilfillan, White, Freeman and Petrakis (1953)	210– 5,600	Man. 6 patients with malignant disease
Reinhardt and Yoffey (1956)	15,100 ± 1,200	Guinea-pigs. Unpublished data. Mean hourly count 20 experiments
Winqvist (1954) . . .	12,750–30,660	6 calves
	2,750– 9,040	4 goats
Brown, Hardenbergh and Tullis (1950) . . .	4,700–19,100	17 dogs

TABLE 45

Cell counts of thoracic duct lymph from the dog

Dog	*Lymphocytes*	*Large mononuclears*	*Transitionals*	*Polymorphonuclear neutrophiles*	*Eosinophiles*	*Unclassified*	*Total counted*	*Remarks*
Bl	452	38	4	0	12	20	526	
Cl	477	85	3	4	79	34	684	
Dl	445	45	0	0	5	7	502	
El	469	17	1	1	0	12	500	
Gl	504	20	0	2	14	10	550	
Hl	405	10	0	63(?)	13	54	545	Only slight blood admixture
Il	431	16	0	7	39	17	510	
Jl	332	18	5	3	4	3	363	
Kl	284	6	1	0	3	9	303	
Ll	478	14	1	1	15	10	519	
Ml	457	20	2	12	37	8	536	
Nl	467	19	0	4	2	0	492	Considerable blood admixture
Pl	257	27	5	8	5	5	307	
Ql	261	13	1	4	4	17	300	
Rl	442	11	1	6	17	32	509	
Sl	484	14	2	0	0	0	500	
Tl	370	12	1	0	5	14	402	
Xl	358	37	4	0	3	0	402	
Yl	371	19	2	3	0	5	400	
Zl	461	24	3	3	0	9	500	
Bt	314	8	3	1	0	9	335	
C_2l	461	17	0	1	14	33	526	
Ft	226	54	2	3	7	8	300	
Total	9,206	544	41	126	278	316	10,511	
Per cent	87·6	5·2	0·39	1·2	2·6	3·0	—	

From Rous (1908*c*)

TABLE 46

Cell counts of thoracic duct lymph from the rabbit

Animal	*Total white blood cells*	*Small lymphocytes*		*Intermediate lymphocytes*		*Large lymphocytes*		*Monocytes*	
		%	*No.*	%	*No*	%	*No*	%	*No*
1	20,466	84	17,291	14·0	2,865	1·0	204	—	—
2	44,960	92	41,363	7·3	3,282	0·1	44	—	—
3	24,270	90	21,843	9·2	2,233	0·5	121	—	—
4	40,728	89	36,247	7·8	3,176	1·9	773	0·15	61
Average of all animals taken together									
	32,606	88	28,693	10	3,260	0·8	260	0·03	15

From Kindwall (1927)

assuming the cells are passing from lymphoid tissue to blood, we have no way of estimating the number of lymphocytes which may enter the blood stream directly in this manner. Not infrequently, in the course of experiments in which the thoracic duct has been cannulated, the blood lymphocytes may show an appreciable increase, despite the fact that large numbers of lymphocytes are being diverted from the blood (Yoffey, 1936; Sanders, Florey and Barnes, 1940). In such cases the animals must be assumed to be in a phase of active lymphocytosis, so that had it not been for the duct cannulation the blood lymphocytes would have been rising to even higher levels. In these cases of lymphocytosis when the thoracic duct is cannulated, the question arises whether the large numbers of lymphocytes entering the blood are doing so indirectly (i.e. are lymph borne) or directly Sanders, Florey and Barnes (1940) incline to the view that one is here dealing with direct entry, though it is difficult to reconcile this interpretation with the findings of Blalock *et al.* (1937), who reported that after complete lymphatic blockage the blood lymphocytes fell almost to zero. Indirect entry would be via the right lymph duct and any other lymphatico-venous communications. The alternative to direct entry would be large scale indirect entry via other lymph channels, but in either case one must assume the occurrence of abnormally active lymphocytopoiesis.

Spleen

In the absence of well-developed lymphatics, cells leaving the spleen must pass directly into the blood, proceeding first of all via the portal vein to the liver. An obvious approach, therefore, to the study of lymphocyte formation in the spleen is through comparative cell counts in the blood of the splenic artery and vein. Morris (1914) and others have found the white cell count in the splenic vein to be markedly higher than that in the artery. Later investigators, however, have been critical of this type of approach. Pearce, Krumbhaar and Frazier (1918) observe: ". . . detailed comparison of the arterial and venous blood of the spleen offers no evidence to indicate, by the methods used, that the spleen has an important rôle in blood formation." Reference has already been made to the tendency for lymphocytosis to develop after splenectomy, and though the interpretation of changes in the level of the blood lymphocytes is beset with difficulty, one obvious explanation could be a compensatory hypertrophy of lymphoid tissue elsewhere. However, direct measurement in guinea-pigs of the thoracic duct lymphocyte output 40 days after splenectomy affords no conclusive proof that such a change is taking place (Reinhardt, Ensell and Yoffey, 1955), though the evidence suggests a trend in that direction.

Thymus

If one accepts the view that the small thymocyte and the lymphocyte are identical—a view which we believe to be correct—the thymus, at least in early life when it is well developed, must also be looked upon as a lymphocytopoietic organ, whatever other functions it may possess. True germ centres are not found there, though nodular formations are sometimes present (Jolly, 1923). The thymic contribution to the blood lymphocytes cannot be measured directly, but it has been inferred indirectly in three ways, namely: (1) by counting the number of mitoses per unit of tissue volume (Kindred, 1940); (2) by counting the mitotic figures in a suspension of cell nuclei (Andreasen and Christensen, 1949); and (3) by measuring the turnover of nucleic acid (Andreasen and Ottesen, 1944)

Kindred (1940), on the basis of mitotic counts in 15- and 20-day-old

are difficult to reconcile with the direct estimation of the cell content of the afferent and efferent vessels of lymph nodes (see Yoffey and Drinker, 1939), though admittedly these latter counts were made on older animals, unless one makes the assumption that two opposing movements of lymphocytes are occurring in lymph nodes, and that while some lymphocytes are continually entering the lymphoid tissue of the nodes from the blood vessels, others are leaving this tissue in the efferent lymph. The evidence of cell multiplication in the lymph node does not accord with this concept; further, the experiments of Andreasen and Ottesen (1944) argue very definitely against such an interpretation.

Andreasen and Christensen (1949) studied activity in isolated nuclei of lymphoid organs (rat), using the citric acid technique to obtain their nuclear suspensions They agreed with Kindred that the greatest mitotic activity was to be found in the thymus and noted that in young animals the thymus has 4–6 times as many mitoses as lymph nodes. But they obtained some perplexing results in their experiments with starvation; this produced a marked decrease of mitotic activity in the thymus associated with atrophy, but on the resumption of feeding the mitotic activity was gradually restored

was accompanied by lymp

and significantly this occu

the recovery. As Andreasen and Christensen themselves observe: "If we consider the thymus as the lymphocyte-producing organ par excellence, it is perplexing that a normal lymphocyte level is obtained and preserved while there is a pronounced atrophy of this organ." It will be recalled

that Sanders and Florey (1940) also concluded that the thymus was not a major source of blood lymphocytes.

Andreasen and Ottesen (1944) made use of the phosphorus content of nucleic acid to throw light on the rate of cell multiplication in the various lymphoid organs. They injected P^{32} into adult rats, and after periods of 2 and 42 hours extracted the desoxyribose nucleic acid from the lymphoid tissues. They then compared the activity of the desoxyribose-nucleic acid phosphorus (DNAP) with that in plasma phosphate, and so obtained data on the rate of desoxyribose nucleic acid formation. They found the nucleic acid turnover was relatively large in all the lymphoid organs, but greatest in the thymus, where it was 2–5 times that found in other lymphoid organs. They further make the very pertinent observation that a high nucleic-acid turnover is convincing evidence in favour of the new formation of lymphocytes within the lymphoid organs and against the large-scale entry into these organs of lymphocytes from blood or lymph. It is also valid evidence against the hypothesis of a lymphocyte circulation.

Bone Marrow

The relationship between lymphocytes, lymphoid tissue and bone marrow is considered in detail in Chapter 7, and we shall here deal more generally with some of the basic questions which present themselves. Does the marrow normally contain either organized lymphoid tissue or scattered lymphocytes, and if so in what numbers? Are the marrow lymphocytes formed in the marrow (myelogenous), or do they enter the marrow from the blood (haematogenous)? If they are myelogenous, are they discharged into the blood in appreciable numbers, or do they remain in the marrow? Finally, whether the marrow lymphocytes are myelo- [illegible] other [illegible] blood [illegible] ase of the spleen, for there are no lymphatics to which they can first obtain access.

An obvious line of attack, as with the spleen, would be to make direct comparisons between the blood of the marrow arteries and veins. Goodall and Paton (1905–1906), investigating digestive leucocytosis, attempted such a comparison and believed they could find more lymphocytes in blood leaving the marrow than in arterial blood. It is clear, however, that they experienced serious difficulties in cell identification, for they comment: "Owing to the fact that in the animals used (dogs and cats) the myelocytes are non-granular, it was impossible to distinguish these cells from large lymphocytes."

A fundamental point to be emphasized is that lymphocytes in mam-

malian bone marrow occur usually as scattered cells, not as organized nodules, the presence of the latter being an exceptional occurrence, mainly if not entirely under pathological conditions (Dominici, 1902; Hedinger, 1907, Longcope, 1906; Oehme, 1909; Aschenheim and Benjamin, 1909; Askanazy, 1915; von Fischer, 1917, Krumbhaar, 1922, Hartwich, 1922, Mayer and Furuta, 1924) Williams (1939), describing his own (human) findings in 302 cases, brings out certain features of interest. He observed lymphoid nodules in 10 per cent of cases under 40 years of age, and in 32 per cent of cases over 40. The margins of the nodules were irregular and merged imperceptibly into surrounding marrow tissue. The nodules were composed almost exclusively of cells indistinguishable from small lymphocytes and there was no evidence of mitosis or of germinal centres. The nodules were associated with active red marrow, and occurred just as frequently in cases where there were inflammatory lesions as not; they were found quite often in cases of sudden death from traumatic shock "The evidence seems to justify the concept that the lymphoid nodules are essentially normal though perhaps variable constituents of the active red marrow of adult individuals."

Kabelitz (1950) found lymphoid nodules present in 12 out of 150 sternal marrows and regarded them as pathological phenomena, associated with disease of the lymph glands and severe blood dyscrasies It is clear from his account that he was dealing with true lymphoid follicles, whereas Williams (loc. cit) emphasized that he was concerned with accumulation of lymphocytes without follicular organization A further point of some significance is that the lymphocyte accumulations described by Williams (1939) and by other workers consist almost exclusively of small lymphocytes Sundberg (1955) notes that in sections of more than 1,500 marrows she refers to "the collections of lymphocytes in the marrow as perivascular lymphocytic aggregations rather than nodules, because they generally lack germinal centres or mitoses visible in sections"

We ourselves (Yoffey and Parnell, 1944, Yoffey *et al* 1951, 1954, Hudson and Yoffey 1952; Harris *et al*, 1954) have examined sections of bone marrow from over 300 guinea-pigs and about 20 rabbits both normal and after the administration of ACTH and various steroids We have never seen any indication of lymphoid nodules or follicles. It is clear that lymphoid nodules may on occasion be found in mammalian marrow, but they are not an integral or essential component. In birds the position may be different. Considerable numbers of lymphoid nodules have been described in the bone marrow of the domestic fowl (Jordan, 1936), turkey (Jordan, 1937), and of the pigeon following splenectomy (Jordan and Robeson, 1942).

But granted that in mammalian marrow one has to reckon with scattered small lymphocytes, in what numbers are these found? The vast amount

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endeavoured to obtain more accurate data (rabbit). They used, with some modification, the technique of Gordon (1939) They took known weights of bone marrow and blood plasma, and found that on shaking for a few minutes the fragments of marrow disintegrated, so that there was obtained a suspension of cells which could be counted in the usual way and from which dry smears and differential counts could be made. The total nucleated cell count (average of 12 cases) was 469,900 per c mm, while the lymphocyte count was 61,000 per c.mm (12 48 per cent), a figure very considerably in excess of the blood lymphocyte level. They calculated that the total lymphocyte content of bone marrow was of the same order of magnitude as the number estimated to be daily leaving the blood.

There is no doubt that these figures, owing to imperfections in technique, were very much on the low side With numerous technical improvements more accurate data were obtained in guinea-pigs. Yoffey *et al* (1951), in 25 normal male guinea-pigs, found a mean lymphocyte count of 142,000 ± 16,000 per c.mm of marrow. Hudson, Herdan and Yoffey (1952) obtained a count of 246,000 lymphocytes per c.mm of marrow, Yoffey *et al* (1954) 322,000 and Harris *et al* (1954) 314,000

Epstein and Tompkins (1943) give a lymphocyte percentage of 11 3 (supravital technique) and 10 2 per cent (sections) in guinea-pig marrow With an absolute count of about 1,400,000 nucleated cells per c mm. of marrow, this would correspond to a lymphocyte count of about 150,000 per c mm, which is practically the count obtained by Yoffey *et al* (1951), and the lowest, incidentally, in the four series of experiments just mentioned.

Taking the figure of 150,000 per c mm. as being the minimal mean concentration of lymphocytes in guinea-pig marrow, we can then proceed, if we know the marrow volume, to calculate the total marrow content of lymphocytes Despite the difficulties arising out of the diffuseness and inaccessibility of the bone marrow, data concerning marrow volume have been obtained by a number of observers. Thus in man Mechanik (1926) calculated that the bone marrow was from 3 4 to 5 9 per cent of the body weight, but that of this amount one half was red and one half yellow marrow in the adult The red marrow was thus between 1 7–2 5 per cent of the body weight, and this is the same kind of figure as was obtained by Nye (1931–1932) in the rabbit, though possibly a little lower than the estimates of Fairman and Corner (1934) in the rat. Our own data (Hudson and Yoffey, 1954) for the guinea-pig are of the same order. The estimations were made in a series of guinea-pigs weighing approximately 400 g and the subsequent calculations of marrow, blood, and thoracic duct lymphocyte content are based on this standard 400 g animal. It is particularly important to use animals of constant age in the investigation of marrow cellularity and response

of material accumulated by clinical haematologists, valuable though it is in so many ways, is unfortunately of no use from a quantitative point of view, since the cell counts thus obtained are from a mixture of bone marrow and blood in varying proportions (see for example Reich and Kolb, 1942); reliable absolute counts of the nucleated cells of the bone marrow are therefore out of the question and only differential counts are possible. But even in the case of differential counts difficulties arise, for a distinction must be drawn between those cells which are common to the marrow and the blood and those which are peculiar to the marrow. In the case of the latter, the blood acts merely as a diluent, and the proportions of the various cells remain unchanged. In the case of the former, however —and the lymphocytes fall into this group—the degree of the dilution becomes an important consideration. As far as the lymphocytes are concerned, it has on occasion been maintained that they are not normally present in the marrow and that their occurrence in sternal puncture material is due to blood contamination (cf. Petri, 1934). However, most workers have been of the opinion not only that they are found in the marrow tissue, but that they are considerably more numerous in this situation than in the blood (e.g. Veeneklaas, 1938), so that even after the marrow has been diluted by blood, there should still be a perceptibly higher absolute count in the sternal puncture fluid than in the blood itself. Blitstein (1944) investigated this point and found that in sixteen cases of sternal puncture the average lymphocyte count in the marrow was 15,200 per c mm., whereas in the blood it was about 3,000. Similar results were obtained when he analysed and compared the data of other workers.

Most haematologists report the lymphocytes to constitute about 10 per cent of the nucleated cells of normal human marrow—e.g. Osgood and Ashworth (1937) 10·6 per cent, Wintrobe (1951) 10 per cent, Osgood and Seaman (1944) 14 per cent, Whitby and Britton (1946) 5–20 per cent, [illegible]

Patterson (1946) state that the lymphocytes constitute 14–21 per cent of the marrow cells, but conclude nevertheless, that they "are not part of the myeloid tissue proper"; they do not, however, elaborate this statement any further. In children the count may be considerably higher (14–57 per cent, Joppich and Liessens, 1937). Doan and Zerfas (1927) in a 4-year-old child found the lymphocytes to be 37·3 per cent of all marrow cells. Little information is available on absolute counts of nucleated cells in human marrow, but on the basis of counts such as those of Isaacs (1937) or Gordon (1939), 10 per cent of lymphocytes would be equal to 50,000–100,000 cells per c mm. of marrow.

The question seemed so important that Yoffey and Parnell (1944)

lymph, with its contained lymphocytes, finds its way once again to the blood. But immediately after the ligature, even of the thoracic duct alone, there is a temporary but effective obstruction to the flow of lymph, whose lymphocytes are prevented from entering the circulation. Under these circumstances the blood lymphocytes show a marked drop, and it is tempting to attach the obvious significance to such an obstruction Thus Lee (1922a) found, after intrathoracic ligation of the thoracic duct in the cat, that the small lymphocytes of the blood decreased 56 per cent (Fig 82) and concluded that the thoracic duct is "the pathway through which at least half of the small lymphocytes reach the circulating blood in the cat".

However, since after ligation of the thoracic duct a variable amount of lymph may still be reaching the blood through the right lymph duct, an obvious improvement on this type of experiment would be to tie both ducts and so obviate this source of error From an experiment such as that of Bunting and Huston (1921) in which 6 hours after ligature of the thoracic and right lymphatic ducts the blood lymphocytes fell from 4,770 to 340 per c.mm, it would seem that the case had been fully proved in favour of the view that the vast majority of the circulating lymphocytes reach the blood via these two ducts But there is one serious objection to such an interpretation, which is that in all these acute experiments the experimental procedure in itself induces a marked fall in the blood lymphocytes and the findings are therefore not as convincing as they would at first sight appear Any prolonged operation gives rise to marked lymphopenia, irrespective of whether the main lymph ducts are cannulated or not, as noted by Sanders, Florey and Barnes (1940) and others. In fact, if an animal is merely anaesthetized and left lying quietly for 2 hours its blood lymphocytes show an appreciable fall (Yoffey, 1933) According to Andreasen and Gottlieb (1947) this post-operative lymphopenia is most marked during the first 24 hours, at the end of which it is still not back to normal, and they attribute this to non-specific factors which the organism eliminates "in the course of the first few days" In part, at any rate, it could be a non-specific "stress" reaction.

Lymphopenia is also produced by cannulating the thoracic duct and diverting its lymph to the exterior (e.g Biedl and von Decastello, 1901; Banti, 1903-1904, Kindwall, 1927) Banti cites experiments of Crescenzi to the effect that whereas simple fistula of the thoracic duct produces a fall of one-half to two-thirds in the blood lymphocytes, if the spleen is first removed the fall is greater—four-fifths to ten-elevenths Here again it is difficult to distinguish between the effects of the diversion of lymph and the operative procedure. Since these experiments were done much additional evidence has accumulated about the output of lymphocytes via the thoracic duct, and this we shall proceed to discuss.

In such animals a marrow volume equal to 2 per cent of the body weight (Hudson and Yoffey, 1954) gives a total volume of 8 c.mm. and a total marrow lymphocyte content of 1200×10^6.

The blood lymphocytes in a series of 17 animals averaged 4,730 per c.mm. (Harris *et al.*, 1954), and with a blood volume of 7 5 ml. per 100 g of body weight (Masouredis and Melcher, 1951) this would mean a total blood lymphocyte content of 142×10^6. On the basis of these figures the ratio of marrow to blood (M B) lymphocytes is about 8 : 1. This is a comparatively low ratio, and in fact, if for this calculation we use the marrow lymphocyte count (322,000 per c.mm.) in the same series of animals as that from which the blood lymphocyte count was obtained (Harris *et al.*, 1954) there would be a marrow lymphocyte population of $2{,}576 \times 10^6$, i e an M:B ratio of about 18 to 1. Before the full significance of these figures can be appreciated they must be compared with data on lymphocyte output.

Ratio of Lymph-borne to other Lymphocytes

We do not know the relative proportion of lymph-borne lymphocytes to those entering the blood directly, but it does not at first sight seem likely that many lymphocytes enter the blood other than via the lymph, for as we have noted in complete lymphatic obstruction (Blalock *et al.*, 1937), where

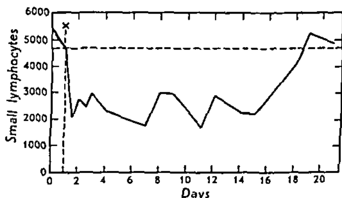


FIG 82—Curve showing the absolute number of small lymphocytes per cubic millimetre of blood in a cat in which the thoracic duct was ligated at X

On the 19th day the number of small lymphocytes had again reached its preligation level The horizontal broken line indicates the level of small lymphocytes at the time of operation

(From Lee, 1922*a*, Fig I, p 251)

the lymph-borne lymphocytes are completely excluded from the blood, the blood lymphocytes fall practically to zero. Partial lymphatic obstruction, effected by ligating the thoracic or the thoracic and the right lymphatic ducts was thought for many years to lead to the same conclusion. In either case a collateral circulation gradually opens and after a time the

TABLE 47

Hourly output of thoracic duct lymphocytes (dog), in each case calculated for a body weight of 10 kg.

No of animal	*Lymphocytes in millions*	*No of animal*	*Lymphocytes in millions*
1	140 9	12	240·4
2	88 5	13	209 6
3	44 7	14	406 1
4	199 9	15	194 4
5	185 5	16	147 7
6	30 2	17	875 4
7	59·3	18	121 5
8	168 2	19	149 8
9	256 6	20	138 7
10	441 5	21	221 5
11	123 5	—	—

Average hourly output (21 experiments) . 211 6 millions
Average output for 24 hours . . . 5,078 millions
In proportion to body weight a human being weighing 70 kg. would have a total daily thoracic duct lymphocyte output of 35,546 millions

From Yoffey (1935–1936)

for lymphocyte production obtained in these experiments must be minimal ones. Since only the thoracic duct was cannulated, lymph from the right lymph duct—which drains the right fore limb, right side of the head and neck, and right half of the thorax—was not included. Moreover, the animals studied were completely anaesthetized and in such animals thoracic duct lymph comes almost exclusively from the abdomen—to some slight extent from the liver, but *mostly from the intestines* Its lymphocytes are, therefore, derived almost entirely from the intestines and mesenteric nodes, there being practically no spontaneous flow of lymph from the extremities or from the head and neck in a quiescent dog. The large numbers of lymphocytes from nodes in the head and neck, therefore, which would ordinarily find their way into the cervical lymph (see Tables 42 and 43) are not represented in lymphocyte output figures such as those of Table 47, except for the data of Reinhardt (1946). As far as lymphocytes

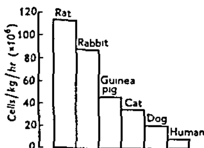


FIG 83—Comparison of figures for thoracic duct lymphocyte output in different animals We are indebted for this figure to Dr W O Reinhardt

The calculations are based on the following papers —

Rat Hungerford and Reinhardt, 1950
Rabbit Sanders, Florey and Barnes, 1940
Guinea-pig Reinhardt and Yoffey 1956
Cat Adams, Sanders and Lawrence, 1945
Dog Yoffey, 1935
Man Bierman, Byron *et al*, 1953

The human data were obtained from diseased individuals and must be treated with reserve

THORACIC DUCT LYMPHOCYTES

Number in Relation to Lymphocyte Output

For the estimation of lymph-borne lymphocytes, and thus of the lymphocytopoietic activity of those lymphoid tissues which possess efferent lymphatics, lymph should be collected from both thoracic and right lymph ducts As a rule, however, observations have been confined to the thoracic duct. Even if both main ducts are cannulated, however, there still remain two as yet unknown factors, namely the extent to which lymphocytes may enter the blood through other lymphatico-venous anastomoses, or the number of lymphocytes which may enter the blood from the lymphoid tissues directly.

As far as the right lymph duct is concerned singularly few data are available. From scanty observations such as those of Warren and Drinker (1942) it is clear that on occasion the lymphocyte content of right duct lymph may be remarkably high Since most of the lymph from both lungs drains into the right lymph duct, the cell content of this lymph would represent presumably the contribution of pulmonary and possibly mediastinal lymphoid tissue. (See also Courtice, Harding and Steinbeck, 1953) Whaler and Widdicombe (1956) found that in rabbits in which the right lymph duct had been ligated the daily lymphocyte output of the thoracic duct rose from $3{\cdot}3 \times 10^9$ to $7{\cdot}4 \times 10^9$. (See also page 351.)

Numerous estimates have been made (see Table 44) of the concentration of lymphocytes in thoracic duct lymph, and the total number of lymphocytes entering the blood via the thoracic duct. The problem was re-investigated by one of us in the thoracic duct lymph of the dog (Yoffey, 1932–1933; 1935–1936) by making counts at 15 minute intervals, using for each count 3 drops of lymph in a small paraffin-lined tube At the end of each hour, the mean of these counts was taken as representing the count in the total lymph collected during that time. Later workers used improved techniques Sanders, Florey and Barnes (1940) allowed a known amount of a 3 per cent sodium citrate solution to mix with the lymph and so collected it continuously for many hours without risk of clotting. In recent years, since heparin has become freely available, it has increasingly been used as an anti-coagulant. The use of polyvinyl or polyethylene plastic tubes as cannulae has also greatly facilitated the collection of lymph over periods of several days (Bollman, Cain and Grindlay, 1948; Mann and Higgins, 1950; Bierman *et al.*, 1953).

Table 47 gives the results obtained by one of us in 21 dogs. It will be noted that the lymphocyte output is subject to considerable variation, as has been noted by other workers (e g Sanders, Florey and Barnes, 1940). In evaluating these results it should be emphasized that the figures

node, but must either have been formed by the endothelium of the lymph capillaries—for which there is no evidence whatever (Clark and Clark, 1932)—or have entered from the tissue spaces Since there is probably no active multiplication of lymphocytes in normal connective tissues, we *must conclude therefore that cells which enter the lymph from the tissue* spaces are cells passing from blood to lymph, and that they are present in the tissues en route from one stream to the other. Clark and Clark (1930) have repeatedly observed the migration of lymphocytes from blood capillaries into the connective tissues, and (1936–1937) from the tissues into lymphatic vessels. A further possibility has been suggested by Osogoe (1943), who reported that an interstitial injection of a suspension of autogenous lymphocytes was removed via the blood, not the lymphatic

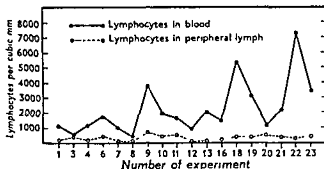


FIG 84—Lymphocyte counts in blood and peripheral lymph in eighteen experiments Lymphocytes in peripheral lymph do not fluctuate with those in the blood The lymphocyte population of peripheral lymph is maintained at a fairly constant level (From Yoffey and Drinker, 1939*a*, Fig 2, p 425)

capillaries. This would imply that peripheral lymph contains no lymphocytes whatever, which is not the case. However, Osogoe (loc cit) quotes some work of Hitachi according to which collections of lymphoid tissue are to be found in the walls of lymphatic vessels, and it is cells derived from this source which are to be found in the lymph If this is correct, then there may not even be a limited circulation of lymphocytes such as has been assumed by Yoffey and Drinker (1939) But while one cannot assert that lymphoid tissue is never to be found in the walls of lymphatic vessels, we believe that it must be very infrequent in mammals, though in birds (see p 24) the position may be different

From the experiments of Yoffey and Drinker (1939*a*) (see Table 40), it is clear that peripheral lymph normally contains some lymphocytes, the mean count in the dog in these experiments being 280 per c.mm. We may observe, incidentally, that lymphocytes in peripheral lymph are maintained at a fairly constant level (see Fig 84); this we interpret as meaning that the lymphocyte population of the *connective tissues is at an equally*

from leg lymph are concerned, the only figures available (see Table 42), which might help us to estimate even grossly the numbers of these cells normally finding their way into thoracic duct lymph, are not wholly satisfactory since in the experiments in which they were obtained the relation of the point of lymph collection to lymph nodes and the main lymphatic pathway is not given.

But granted that the lymphocytes in thoracic duct lymph are derived mainly from the abdomen, is it possible to state with precision whether any special region is involved? Sanders and Florey (1940) showed in the rabbit that after removal of the pancreas of Aselli a marked fall in the blood lymphocytes occurred, though a substantial number still remained. Though they also removed Peyer's patches and the sacculus rotundus—the latter containing a considerable amount of lymphoid tissue—they did not subsequently measure the thoracic duct lymphocyte output in these animals. More recently Mann and Higgins (1950) showed in rats that virtually the same figures for lymphocyte output were obtained whether they cannulated the thoracic duct in the neck, or the main intestinal lymph trunk in the abdomen. They showed that relatively few cells entered the thoracic duct from liver lymph and felt justified in concluding that "... with the animal unanaesthetized and freely feeding but limited in its activity, the intestine appears to be practically the exclusive source of the lymphocytes in the lymph". Though they do not mention the pancreas of Aselli, it would appear from their description that their "intestinal" lymph drained this also.

Numbers in Relation to Peripheral Lymph: Circulation of Lymphocytes

Granting the assumption that large numbers of lymphocytes are daily entering the blood via the thoracic duct, the question arises as to whether these are newly formed cells, or whether they are cells which have entered the lymph from the blood stream to which they are now returning. Nothing is known of any possible function that such a circulation of lymphocytes might subserve.

For the problem under consideration it is necessary to examine lymph which has not yet traversed a node or any organized mass of lymphoid tissue, since lymph which has made such a passage contains many lymphocytes, and one cannot say whether these cells were present in the lymph before it reached the node or whether they were taken up from the node during transit. Several observers have shown that large numbers of cells are added to lymph when it passes through a node (Heyfelder, 1852; Brücke, 1854; Goodall and Paton, 1905–1906; Florey, 1926–1927; Haynes and Field, 1931; Baker, 1932–1933).

Cells present in peripheral lymph cannot have been derived from a

the observations of Bierman and his co-workers, lymph was draining freely for periods ranging from 2–11 days, without any obvious effect on the condition of the patients or the composition of the blood, though in the case described by Courtice, Simmonds and Steinbeck the fistula was associated with lowering of the plasma proteins and poor general condition. In the animal experiments of Mann and Higgins the thoracic duct fistula was maintained in several instances for 6 days, in those of Shrewsbury and Reinhardt for 4 days, and in those of Glenn, Bauer and Cresson for periods up to 12 days In all these animal experiments the thoracic duct lymphocyte output showed a progressive decrease, while at the same time there was a fall in the blood lymphocytes. At first sight these experiments might appear to support the hypothesis of a lymphocyte circulation, but in fact, if one compares the lymphocytes collected in the thoracic duct fistulae with those disappearing from the blood, the former greatly exceed the latter

Reinhardt, using unpublished haematological data from the experiments of Shrewsbury and Reinhardt (1952), has been kind enough to make some calculations for us on these lines, from which it appears that in animals whose fluid intake consisted of 1 per cent NaCl, in the 4-day fistula experiments 75 times more lymphocytes were collected in thoracic duct lymph than were lost from the blood, as judged by a comparison of the blood lymphocytes at the beginning and end of the experimental period In animals given water to drink the ratio was 14 . 1. Ratios such as these make it very difficult to accept a simple recirculation hypothesis. The progressive fall in lymphocyte output has not yet been accounted for, and Mann and Higgins (1950) attempted to prevent it by re-injection of the fistula lymph, but without success Glenn, Bauer and Cresson (1949) examined histologically the popliteal nodes of 2 dogs before and after a fistula had been established, but could find nothing to indicate diminished lymphocytopoiesis

By way of summary we may say therefore that circulation of lymphocytes between blood and lymph occurs on only a small scale, and that the majority of the lymphocytes which enter the blood through the main lymph ducts are newly formed cells. This view is based upon (1) the evidence of new cell formation in lymphoid tissue, as observed directly by mitotic counts, and indirectly through the P^{32} turnover associated with the synthesis of desoxyribonucleoprotein. (2) The increased activity of lymphoid tissue on feeding and its diminished activity when food is withheld (3) The fall in the blood lymphocytes after lymphoid tissue extirpation and the subsequent regeneration of new lymphoid tissue (4) The results of prolonged drainage of the thoracic duct when the lymph contains many more lymphocytes than can have been derived from the blood (5) The low lymphocyte count of peripheral lymph

constant level. To the extent that some lymphocytes are always entering peripheral lymph from the blood, a circulation of lymphocytes may be said to take place. (See also Allen, 1945.) But when one compares peripheral and central lymph, these circulating lymphocytes are found to be only a small fraction of the total number entering the blood. The average lymphocyte count in the thoracic duct lymph of the dog (24 experiments, Yoffey, 1932–1933) was 9,040 per c mm.; the average count in peripheral lymph (Yoffey and Drinker, 1939*a*), 280 per c.mm In these experiments, therefore, of the lymphocytes entering the blood via the lymph, 1 in 32 might be regarded as returning to the blood after having entered the lymph, while the other 31 were newly formed cells.

Rous (1908*a*) gives a considerably lower figure for thoracic duct lymphocytes, owing no doubt to the fact that his animals were starved for 24 to 48 hours before the experiments; as has already been pointed out (Chapter 5), starvation often has a depressant effect on lymphocyte formation. The mean total white cell count (14 experiments) in Rous's series was 4,720 per c mm. Even with these lower figures, the proportion of peripheral to central lymphocytes would be about 1 to 16.

Sjovall (1936) does not accept this interpretation of a very limited circulation of lymphocytes but advances the analogy of a particle-containing lake He argues that if at any given time there are more particles in the water leaving the lake than entering it, this does not prove that the particles were actually formed in the lake, but merely that they were present in it when water passed through. This objection no doubt could be applied to a single observation; but if it can repeatedly be shown that there are always far more particles leaving the lake than entering it, then the analogy breaks down Furthermore, the lake of this analogy corresponds to lymph nodes and lymphoid tissues, whose lymphocytes are not inert particles but cells undergoing active multiplication. (See also Fichtelius, 1953) Farr (1951) suggests that lymphocytes might pass from the blood to the lymphoid tissues direct, without first entering the lymph stream.

The hypothesis of a lymphocyte circulation may be put to the test indirectly in several ways If most of the blood lymphocytes enter the lymph, one would expect the lymphocyte content of these two fluids to run parallel This, however, is not the case. Fig 84 shows that there is no constant numerical relationship between lymphocytes in blood and in peripheral lymph. (See also Bierman, Byron *et al.*, 1953.)

In recent years new techniques, involving the use of plastic cannulae, have made possible prolonged drainage of lymph from the thoracic duct (Mann and Higgins 1950, and Shrewsbury and Reinhardt, 1952, both working with rats; Glenn, Bauer and Cresson, 1949, dog; Courtice, Simmonds and Steinbeck, 1951, man; Bierman *et al*, 1953, man). In

been made in various ways. One of the most obvious of these is to make quantitative comparisons between thoracic duct and blood lymphocytes This is a useful approach to the problem, though it is one which is open to criticism on several scores. As an illustration of this type of approach we may take the data of Yoffey (1936) Figures for thoracic duct lymphocyte output have been presented in Table 47, and it is apparent that the output in dogs shows great variation. Is this variability permanent? Does one dog normally and regularly have only 30,200,000 lymphocy [illegible] duct into the blood, while another [illegible] s that amount? It seems unlikely, [illegible] l be so. In experiment 17

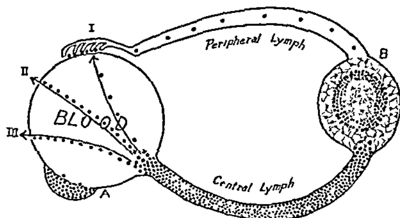


FIG 85 —Diagram illustrating features of the life cycle of the lymphocyte

A, lymphoid tissues from which lymphocytes pass directly into the blood, *B* lymphoid tissues from which lymphocytes first enter the lymph stream, in which they are then conveyed to the blood

(From Yoffey and Drinker, 1939*a*, Fig I, p 424)

(Table 47), for example, an assumption of constancy of cell content in the thoracic duct of individual animals would mean a lymphocyte output [illegible]

circulation

The number of lymphocytes in the blood may be calculated approximately from the count per c mm. and the estimated blood volume. Mayerson (1930) in 60 dogs found the average count of small and large mononuclears in the blood to be 2,680 per c.mm. If we assume that all these mononuclears were lymphocytes—which they almost certainly were not—then any error introduced in calculations of the daily replacement of blood lymphocytes will lead to under- rather than to overstatement. The

Under these circumstances, the only way in which the facts could be made to fit in with the hypothesis of a lymphocyte circulation would be on the assumption that lymph nodes are capable of concentrating lymph. If this assumption were fact, the protein content of lymph leaving nodes would be much higher than that of lymph entering nodes; such is not the case. Many experiments have shown that lymph protein, peripheral and central to nodes, is identical in amount. There is thus no direct evidence for absorption of water in nodes. It may be held that fluid concentration is obtained by the simultaneous absorption of water and of lymph protein, so that the concentration of the lymph protein remains constant, but the lymphocytes per c.mm of fluid increase in number. If, however, the blood capillaries of lymph nodes are capable of large scale absorption of protein, they are not only unique members of the blood capillary system, but at the same time display a miraculous ability to absorb protein so equitably as to make afferent and efferent lymph precisely equal in protein content.

Numbers in Relation to Blood Lymphocytes

Large numbers of lymphocytes regularly enter the blood via the thoracic duct Since the number of lymphocytes in the blood remains fairly constant, it is evident that lymphocytes must be disappearing from the blood at the same rate as they are entering it. In the following pages we shall discuss various aspects of this relationship between thoracic duct and blood lymphocytes. It will help to clarify the problem if we outline it schematically (Fig. 85).

Lymphocytes are formed in the various lymphoid tissues, and may enter the blood either directly, without first passing into the lymph (Fig. 85—*A*), or via the lymph stream, *B* The fate of the lymphocytes once they have entered the blood is unknown It has been suggested that they may disappear from the blood in one of four ways: (1) They may pass into the connective tissues (Fig. 85—*I*), thence into the lymphatic vessels and back into the blood. The experimental evidence indicates that this explanation would account for the disappearance of only one-sixteenth to one-thirty-second of the blood lymphocytes. (2) They may pass through the mucous membrane into the lumen of the alimentary canal and so be lost to the animal (Fig 85—II). (3) They may be filtered out of the blood stream by the bone marrow (Fig. 85—III), where they have been held to constitute the essential cells for blood formation (4) They may grow old and degenerate while in the blood. Of these possibilities, *1, 2, and 3 have been indicated in the diagram; 2, 3, and 4* will be considered in detail in subsequent sections.

An important factor in the situation is the length of time during which lymphocytes remain in the blood. Attempts to answer this question have

been made in various ways. One of the most obvious of these is to make quantitative comparisons between thoracic duct and blood lymphocytes. This is a useful approach to the problem, though it is one which is open to criticism on several scores. As an illustration of this type of approach we may take the data of Yoffey (1936). Figures for thoracic duct lymphocyte output have been presented in Table 47, and it is apparent that the output in dogs shows great variation. Is this variability permanent? Does one dog normally and regularly have only 30,200,000 lymphocytes per hour passing via the thoracic duct into the blood, while another has 875,500,000—almost thirty times that amount? It seems unlikely, for several reasons, that this should be so. In experiment 17

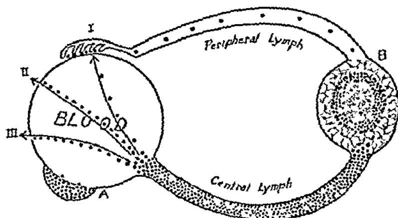


FIG. 85.—Diagram illustrating features of the life cycle of the lymphocyte

A, lymphoid tissues from which lymphocytes pass directly into the blood; *B*, lymphoid tissues from which lymphocytes first enter the lymph stream, in which they are then conveyed to the blood

(From Yoffey and Drinker, 1939*a*, Fig. 1, p. 424)

(Table 47), for example, an assumption of constancy of cell content in the thoracic duct of individual animals would mean a lymphocyte output during the 24 hours in this particular dog of 21,000,000,000, or 4·15 times the average figure. The significance of this figure may best be appreciated when it is compared with the total number of lymphocytes in the circulation.

The number of lymphocytes in the blood may be calculated approximately from the count per c.mm. and the estimated blood volume. Mayerson (1930) in 60 dogs found the average count of small and large mononuclears in the blood to be 2,680 per c.mm. If we assume that all these mononuclears were lymphocytes—which they almost certainly were not—then any error introduced in calculations of the daily replacement of blood lymphocytes will lead to under- rather than to overstatement. The

blood volume may be calculated from the body weight. Smith, Arnold and Whipple (1921), after a careful comparison of the various methods on the same group of dogs and after an analysis of the possible sources of error, conclude that the blood volume in dogs is 9·2 ml per 100 g of body weight. A 10 kg. dog would have a blood volume of 920 ml., and would contain 2,465,000,000 lymphocytes. Since the blood lymphocytes remain constant over a period—so that the same number must be entering the blood as are leaving it—lymphocyte production on a scale as vast as that found in experiment 17 (Table 48) would be sufficient to replace the *blood lymphocytes in the average dog 8·5 to 10 times daily. Actually the* blood lymphocytes in this experiment were above the average, being 6,170 per c.mm ; but even at this higher level they were being replaced 3·71 times per day.

The dog used in experiment 17 had the highest lymphocyte output of the animals studied. The average figure for the series would appear to be a fairer one to use and probably gives a truer picture of thoracic duct lymphocyte production over a period. On the basis of this figure, the average daily output of lymphocytes from the thoracic duct of a 10 kg. dog is 5,078,000,000 This means that the blood lymphocytes are normally replaced 2·06 times per day and we may refer to this as the D.R.F. (*Daily Replacement Factor*). *If we adopt the later figure of* Courtice (1943) for the blood volume in the dog, namely 7·9 ml per 100 g. body weight, the D.R.F. would be a little higher, 2·38. The lymphocytes, on this basis, remain in the blood for only 12 hours or a little under *Other* figures for the D.R.F. are: Sanders, Florey and Barnes (1940), 0·5–3·5 in the cat, 5·0 in the rabbit; Adams, Saunders and Lawrence (1945), 2·2 in the cat; Reinhardt (1946), 2·05 in the rat; Mann and Higgins (1950), 5·0–6·0 in the rat; Courtice, Simmonds and Steinbeck (1951), 1·0 or more in man On the whole, one has the impression that in smaller animals both the number of lymphocytes in lymph and the D.R.F. tend to be higher than in large animals, but the point cannot be made with any degree of certainty. As far as man is concerned, the data available (Courtice, Simmonds and Steinbeck, 1951; Bierman *et al.*, 1953) appear to indicate a lower concentration of lymphocytes in thoracic duct lymph *than is to be found in most animals*, but the data were obtained from patients who were ill and may be below the normal figure.

It should be emphasized that estimates of the D.R.F. based solely on thoracic duct lymphocyte output are minimal. *To calculate the true* figure we should need to know the number of cells entering the blood through channels other than the thoracic duct and it might prove to be [illegible] It seems certain

noted it may be sufficient to reverse the lymphopenia which is usually associated with thoracic duct cannulation In these cases it may in fact exceed the thoracic duct output unless we assume some interference with the escape of lymphocytes from the blood—but even if it merely equalled it, the figures which we have just given for the D.R F. would need to be doubled. If this were to hold good in all cases—and of this we are by no means sure—it would mean that in the rat and rabbit we would have a D.R.F. of about 10, with the lymphocytes remaining in the blood for about 2½ hours.

Of interest in this connection are the experiments already noted of Whaler and Widdicombe (1956). They first estimated thoracic duct lymphocyte output in a number of rabbits, and obtained a figure of 3.3×10^9 cells per day, agreeing with the data of Sanders, Florey and Barnes (1940). After ligating the right lymph duct, the thoracic duct output rose to 7.4×10^9 cells per day, and this increase they attributed to the fact that lymphocytes which would normally have been entering the blood via the right lymph duct were now passing through anastomotic channels to the thoracic duct They calculated that, making allowance for the subclavian and cervical duct output in addition, the D.R.F in the rabbit was about 12.0, and in the rat 24.0

That lymphocytes remain in the blood for a short time only may be shown in another way. By ligating the thoracic and right lymphatic ducts, or by cannulating the thoracic duct and diverting its lymph from the blood, one prevents a large number of newly formed lymphocytes from entering the blood, but does not presumably interfere with the mechanism whereby lymphocytes already present in the blood leave it Under these circumstances, the blood lymphocytes fall rapidly (Table 48) Thus, in an experiment of Bunting and Huston (1921) in the rabbit, ligation of the thoracic and right lymphatic ducts was followed by a fall in the blood lymphocytes from 4,770 to 340 per c mm in 6 hours This is significant even if it is not entirely consequent upon the ligation of the ducts, but is in addition due to the operative procedure itself—provided that it is not associated with marked lymphocytolysis. This result suggests that in the rabbit the lymphocytes remain in the blood for an even shorter time than they do in the dog. It is interesting to note that under these circumstances though the lymphocytes fall so markedly, they never disappear from the blood completely Though the main inflow to the blood is checked, some lymphocytes are still entering, either directly from the lymphocytopoietic tissues or through small additional lymphaticovenous communications, the ultimate enlargement of which permits the return of the blood lymphocytes to normal. Thus, in an experiment of Biedl and von Decastello (1901), after ligation of the thoracic and right lymphatic ducts, the blood lymphocytes fell from 2,147 to 1,146 in 6 hours, to

452 after 1 day; to 494 after 2 days; and after 4 days had risen to 1,523 per c.mm, suggesting perhaps the development of a new channel of entry into the blood, though other explanations could also be advanced

In considering variations in thoracic duct lymphocyte output it should be noted that the concentration of lymphocytes may undergo either progressive increase or diminution during the course of an experiment

TABLE 48

Effect on blood lymphocytes of ligating thoracic duct, or of ligating thoracic duct with right lymphatic or both cervical ducts

Author	*Animal*	*Blood lymphocytes per cubic millimetre* *Before ligature*	*After ligature*	*Time after ligature of making blood count*	*Remarks*
Lee (1922*a*)	Cat	4,689	2,050	12 hours (Approx)	Thoracic duct ligated (see fig. 82)
	,,	3,139	1,373	12 hours (Approx.)	Thoracic duct ligated (see fig. 82)
Biedl and von Decastello (1901)	Dog	2,147	1,146	6 hours	Thoracic and right lymphatic ducts ligated
		—	452	1 day	
		—	494	2 days	
		—	1,523	4 ,,	
Bunting and *Huston* (1921)	Rabbit	2,890	1,064	5 hours	Thoracic and right lymphatic ducts ligated
	,,	4,770	340	6 ,,	Thoracic and both cervical lymph ducts ligated
Davis and *Carlson* (1909–1910)	Dog	4,300	3,340	8 ,,	Thoracic and cervical lymph ducts ligated in both animals
	,,	—	2,460	20 ,,	
	,,	2,240	1,940	18 ,,	

(Yoffey, 1935–1936; Sanders, Florey and Barnes, 1940) in which the rate of lymph flow remains unaltered Such variation is possibly to be correlated with the cyclic changes which occur in lymphoid tissue, demonstrated histologically by Conway (1937).

FATE OF BLOOD LYMPHOCYTES

Disintegration in the Blood

The quantitative relationship thus established between thoracic duct and blood lymphocytes would hold good whether the lymphocytes disintegrated in the blood or left the blood stream. For the further consideration of the fate of the blood lymphocyte, it is necessary to determine whether large scale disintegration does actually occur. There is no doubt that some disintegrating lymphocytes [illegible]

in the blood does not seem to be the fate of the majority of the blood lymphocytes.

Bunting and Huston (1921) counted the lymphocytes in the freely flowing blood of the rabbit and then isolated a section of the jugular vein between double ligatures. Cell counts in this jugular blood were made 6 hours later in some animals and 24 hours later in others. "In the stained film from the jugular blood, the most striking feature is the fact that the lymphocytes show no tendency towards disintegration but are as sharply stained and clear-cut in the differentiation of nucleus and protoplasmic rim as in films from the freely circulating blood. We believe that the experiments, though faulty, indicate that there is not the rapid disintegration of the cells which would be necessary to explain the rate of their disappearance from the blood when the supply is cut off. They must leave the blood stream"

Wiseman (1931–1932) concluded that "at least a portion of the so-called lymphocytes arise, mature, function and die as lymphocytes without differentiating into other cell types" Whether one accepts this statement or not depends upon the size of the "portion". It seems clear from the general trend of Wiseman's paper that he interprets the word portion somewhat liberally. He described in the circulating blood an end stage of the lymphocyte, consisting—in the supravitally stained preparation—of an apparently free nucleus without cytoplasm or definite structure of its chromatin. The nuclear outline is round and very sharp, resembling that of cells treated with acetic acid. "This form has been found up to about 5 per cent of the total lymphocytes." But in view of the rapid rate of disappearance of lymphocytes from the blood, far more of these end stages should be seen if senescence and degeneration in the blood stream were the main reason for lymphocyte disappearance In the experiments of Bunting and Huston, cells which would have disappeared from the blood in 6 hours were shown not to have undergone degenerative changes even after 24 hours. Furthermore, in tissue cultures, thoracic duct lymphocytes which are about to enter the circulation and die within 12 hours, if Wiseman's interpretation is correct, possess a viability of several days This is admitted, even by those workers who have been unable to detect any sign of further growth and differentiation In this connection the experiments of Ebert, Sanders and Florey (1940) are of especial interest. Using the transparent chamber technique in the rabbit's ear, they found that lymphocytes from a fragment of popliteal lymph node (autogenous) remained alive and motile in the neighbourhood of blood vessels in one case for 7 days, in another for 8 days, while in a third many lymphocytes were still present after 26 days. That cells which can live for this length of time should regularly enter the blood stream and disintegrate within a few hours is difficult to believe.

Excretion into Lumen of Alimentary Canal

Bunting and Huston (1921), after noting the rapid disappearance of lymphocytes from the blood and finding no evidence of their destruction while in the blood stream, suggested that they were excreted into the lumen of the alimentary canal. On histological examination of the intestine, these investigators found large numbers of lymphocytes in the epithelium close to the surface and some free lymphocytes in the lumen of the intestine close to the mucosa.

Hellman (1932) and Stenqvist (1934) could not confirm the finding by Bunting and Huston that there is an extensive migration of lymphocytes through the mucous membranes Hellman denied the occurrence of this in the tonsillar epithelium, in particular. (See also Ikeda, 1950–1951; Isaacs and Danielian, 1927.) Stenqvist concluded that if the intestines are handled carefully and not subjected to excessive manipulation, evidence of lymphocytic migration through the mucosa is difficult to obtain. He nevertheless assigned an antibacterial function to the lymphocytes in the intestinal wall and found confirmation of this in the fact that lymphocytes are absent from the mucous and submucous coats of the intestines in bacteria-free guinea-pigs. On the other hand, in newborn animals large numbers of lymphocytes are present in the intestinal wall even before bacterial contamination occurs.

Kelsall (1946), working with hamsters, found that there were many lymphocytes present both in and between the intestinal epithelial cells, but that the overwhelming majority (96 4 per cent out of 6,595 counted) were in the deeper portion of these cells, between the nucleus and the basement membrane. This would suggest that though the lymphocytes in this situation are very close to the lumen of the intestine, they do not quite reach it. She further found no evidence of lymphocyte dissolution or fragmentation, but on the contrary thought it possible, though she did not examine the point in detail, that there might be a transformation of lymphocytes into plasma cells which then migrated into the stroma of the villi Measurement showed that the nuclei of small lymphocytes in the epithelium were the same size as nuclei of small lymphocytes in adjacent Peyer's patches. As far as fragmentation was concerned, Kelsall, because of the high thymonucleic acid content of lymphocytes, thought that if they fragmented the epithelial cells should contain particles rich in thymonucleic acid; however, staining for thymonucleic acid by the Feulgen method failed to demonstrate these.

Andrew and Sosa (1947) observed many lymphocytes undergoing mitosis within the cells of the intestinal epithelium of mice, thus confirming the earlier work of Andrew and Andrew (1945). Nevertheless, in spite of the not infrequent mitoses, they thought that on the whole the histo-

trachea (Andrew and Burns, 1947), but in this situation a regular migration into the lumen was considered improbable.

It is clear then that though many lymphocytes are found in the intestinal mucosa close to the lumen, this in itself does not prove that they actually enter the lumen. It is obviously desirable to have information about the number of lymphocytes actually present *in* the lumen. In this connection the experiments of Jassinowsky (1925) are frequently quoted, but they are in fact singularly unconvincing. In 6 rabbits, 2 dogs and 1 cat, he made an incision in the abdominal wall and brought to the exterior a loop of intestine which was kept moist with warm normal saline. Into either end of the loop he tied a cannula, connected with a rubber tube and funnel, and into one funnel poured 20 ml of warm saline. By alternately raising and lowering the funnel at either end, the loop was irrigated 6–9 times every 5 min for ½–1 hr., and the washings examined for cells. These washings contained varying numbers of lymphocytes, but also a considerable number of desquamated epithelial cells. It is not altogether surprising that there was a fair degree of correlation between the extent of the desquamation and the number of lymphocytes. Jassinowsky then endeavoured to find out whether the lymphocytes which had (so he believed) migrated through the intestinal epithelium came from blood vessels or lymphoid nodules. Accordingly, in one animal he ligated all the blood vessels supplying an intestinal loop and then made further irrigations. He found that again (except for the appendix) lymphocytes and epithelial cells increased in the washings. The increased discharge of epithelial cells he did not attempt to explain, but that of the lymphocytes he attributed to "emigration".

How many lymphocytes are there in the intestinal mucosa—apart, that is, from the solitary and the aggregated nodules? The only information available has been provided by Kindred (1942), who concluded that the intestinal epithelium contained three times as many lymphocytes as the circulating blood. From a quantitative point of view, therefore, this ratio is not incompatible with the hypothesis of the intestinal elimination of lymphocytes.

Apart from the direct enumeration of lymphocytes in the intestinal lumen, an indirect method of experimental approach was adopted by Erf (1940), who subjected rabbits to gastro-enterectomy and then observed the rate of disappearance from the blood of lymphocytes already in it, as well as of others which were injected intravenously. He found that: "Viable lymphocytes, either autogenetic or heterogenetic, administered

intravenously to either normal or gastro-enterectomized rabbits, disappear very rapidly from the circulating blood. Excretion of lymphocytes through the mucosa of the stomach and intestines is not the only mechanism responsible for lymphocyte destruction." Apart from the assumption that the only purpose for which lymphocytes leave the blood is to be destroyed, this is an interesting contribution to the problem of the fate of the blood lymphocyte, more clear-cut than the experiments on enterectomy and the blood lymphocytes described by Yoffey (1942).

It should, however, be noted that experiments such as those performed by Erf, while they show that lymphocytes may rapidly leave the blood in large numbers even after the removal of most of the alimentary canal, do not directly disprove the occurrence of intestinal elimination

Since the lymphocyte is not phagocytic the advocates of the intestinal elimination hypothesis have suggested that its function is to affix toxins (e g Bunting and Huston, 1921 ; Moor and Newport, 1939)

Sjovall (1936) also resorts to the hypothesis of toxin fixation to support his general theory of a synergic action between the circulating lymphocytes and the fixed reticulum cells of the lymphoid tissues. He suggests that lymphocytes pass out of the blood stream into the connective tissues, take up endogenous and exogenous toxins, and transport them to the lymph nodes, where they are neutralized or destroyed by the fixed reticulum cells. The lymphocytes thus freed of toxins re-enter the blood to start the toxin-fixing cycle once more. The recent work of Coons and his collaborators (see p. 275) has however shown that lymphocytes do not take up more than traces of toxin or antitoxin.

Saliva may contain at times an appreciable number of leucocytes, but from observations such as those of Isaacs and Danielian (1927) and Ikeda (1950–1951), it is clear that the bulk of the salivary corpuscles, with some exceptions, are neutrophile granulocytes. Isaacs and Danielian (1927) found this to be so even in cases of lymphatic leukemia, but they did find increased numbers of salivary lymphocytes in patients with pyorrhoea Ikeda (1950–1951) comments · "Lymphocytes never occurred in saliva in one-seventh of all cases." But the cells, if present, ranged from 0 4 to 17 per cent, the latter figure being found in a patient with oral inflammation. It is of interest that in the vagina also the bulk of the leucocytes are polymorphs ; and lymphocytes, monocytes and eosinophiles together never constitute more than 4 per cent of the total cell count (Gutmacher, 1926).

It is perhaps appropriate at this point to refer to the suggestion by Andrew and Andrew (1949) of a novel fate for the blood lymphocyte They point out, after a survey of the literature, that : "The impression that the mitotic divisions present in epidermis are not numerous enough to account for the amount of cell renewal necessary has been expressed

by various authors." But if this be true, either the epidermal cells must multiply by amitotic division, for which there seems to be no satisfactory evidence, or else one must postulate "the addition to the epidermis of non-epidermal cells with subsequent differentiation of such cells to become epithelial in character". On the basis of their histological studies, Andrew and Andrew conclude that lymphocytes are of constant occurrence in the normal epidermis of the rat and of man, in which they constitute 1–4 per cent of the cells of the stratum germinativum; they are not found in the layers superficial to this These lymphocytes do not degenerate, but undergo changes both in their nucleus and cytoplasm to become finally transformed into epithelial cells. Such an origin for the cells of the epidermis would account both for the infrequent occurrence of mitotic figures in the stratum germinativum, and for the inability to demonstrate any other mode of rapid regeneration.

However, Andreasen (1952) was unable to find in the epidermis any evidence of lymphocyte transformation, and concluded that "the ultimate fate of the epidermal lymphocyte is degeneration". He also thought that "the epidermis is one of the disposal areas for the lymphocytes of the body, and it seems probable that released enzymes or breakdown products of the lymphocytes may be of importance in the metabolism of the epidermal epithelium".

Lymphocytes in Connective Tissue

Of the three main groups of scattered lymphocytes, our ignorance is perhaps most profound concerning those in the connective tissues of the body. There is no doubt that a small number of lymphocytes is continually escaping from the blood capillaries all over the body (though only to a minor extent in the nervous system) into the connective tissues, and thence finding their way into lymphatic capillaries It is conceivable that some multiplication of lymphocytes may occur in the connective tissues, though in connective tissue spreads mitosis of lymphocytes is exceedingly infrequent Nevertheless, the possibility of such mitosis cannot be completely excluded, more especially in pathological conditions, where the question of the origin of the lymphocytes which may appear on occasion in such large numbers has long been a source of controversy Some of the key references to the literature, on the haematogenous or histogenous origin of the lymphocytes in inflammation, may be found in the papers of Kolouch (1939) and of Rebuck and Crowley (1955)

No quantitative study of connective tissue lymphocytes has been made. No investigator has yet undertaken the heroic task of counting lymphocytes in serial sections of an entire animal, or even of trying to obtain an approximate idea of the magnitude of the lymphocyte population of the connective tissue by thus examining even a limited mass of tissue.

Allen (1945) argued that the peritoneum could be regarded as a greatly enlarged tissue space and the lymphatics of the diaphragm as the lymph vessels that drained this space. He then made cell counts of peritoneal fluid and diaphragmatic lymph, and found a consistently higher count—of both lymphocytes and other cells—in the former. He concluded, therefore, that lymphatic endothelium offers a certain degree of resistance to the passage of lymphocytes, and interpreted this to mean that the essential function of the lymphocyte is discharged while in the connective tissue This is an interesting hypothesis, but is open to a number of objections. Apart from the question as to whether it is safe to infer that the conditions found in the peritoneal cavity are applicable to the connective tissues generally, it must be remembered that the lymphocyte is an actively motile cell and presumably does not function as an inert particle Unless one postulates a chemotactic attraction of lymphatic endothelium for the lymphocyte—and so far there is no evidence of any chemotactic stimulus for lymphocytes (McCutcheon, 1955)—there will be as many lymphocytes in the connective tissues moving away from lymphatic capillaries as towards them. The lymphocyte population of the connective tissues will therefore represent, in part at any rate, a dynamic and not a static equilibrium It is not simply a question of the steady passage of lymphocytes through these tissues in one direction only, namely, from blood to lymphatic capillaries ; the occasional movement of lymphocytes away from the lymphatic endothelium would account very largely for " discrimination ".

Passage into Bone Marrow

Possible passage of lymphocytes into bone marrow, together with the problem of the developmental potentialities of the lymphocyte, is discussed at length in Chapter 7

THE BLOOD SPAN AND THE LIFE SPAN OF THE LYMPHOCYTE

In an attempt to throw further light on the fate of the lymphocyte once it enters the blood, numerous experiments have been performed involving a variety of techniques, including the transfusion of lymphocytes with and without some form of labelling, parabiotic preparations, and cross-circulation experiments. In addition, lymphocytes have been labelled—by radio-active isotopes or fluorescent dyes—in the intact organism. Though our main interest is in the lymphocyte, it will be convenient in describing these experiments to refer also to some of the results concerning other leucocytes. It is important, in considering the data about to be presented, to distinguish clearly between the total length of life of the leucocytes (life span), and the length of time they spend in the blood stream (blood span).

Simple Transfusion

One of the earliest observations was made by Minot and Isaacs (1925). A patient with advanced lymphosarcoma with a leucocyte count of 6,400 per c.mm. (lymphocytes 15·5 per cent) was given 450 ml of blood from a patient with chronic lymphatic leukaemia, with a blood leucocyte count of 89,000 (lymphocytes 95·6 per cent)

Immediately after the transfusion, there was a fourfold increase in the blood lymphocytes of the recipient, but within 35 minutes they had dropped almost to their pre-transfusion level. There was nothing to indicate that the transfused lymphocytes were destroyed in the circulation.

In an experiment such as this, the transfused cells were without doubt predominantly lymphocytic, and the first question which arises is whether their reactions could be considered identical with those of the recipient's lymphocytes If they could, these leukaemic lymphocytes would have a blood span of about half an hour The further question then arises whether this would hold good for non-leukaemic cells

Weiskotten (1930) gave benzol to rabbits and examined the bone marrow He found that when the marrow was no longer producing neutrophiles they disappeared from the blood in 3–4 days, suggesting that the neutrophiles remained in the blood a good deal longer than the lymphocytes. However, Kindred (1942) on the basis of a series of calculations of the volume of myeloid and lymphoid tissues, the proportion of mitotic cells, the estimated length of the mitotic cycle, and the cells in the blood stream, concluded that in the blood of the rat the granulocytes were replaced six times as frequently as the lymphocytes. Van Dyke and Huff (1951) recalculating Kindred's data concluded that the blood span of the granulocytes was 30 minutes, of the lymphocytes 188 minutes

Van Dyke and Huff (1951) by quite different methods obtained results of the same order as Kindred (1942) They used parabiotic rats, one of which was exposed to radiation (950r) sufficient to destroy the greater part of its haemopoietic tissues From data available concerning the amount of blood passing through the anastomosis between the two animals, and the leucocytes received by the irradiated from the non-irradiated member, they concluded that " the average survival time of the mononuclear leucocytes in the blood of an irradiated parabiotic rat was 170 minutes while the average survival time of the polymorphonuclear leucocytes was 23 minutes ".

Farr (1951), employing a technique devised by De Bruyn, Robertson and Farr (1950), injected intravenously into rabbits suspensions of autogenous lymphocytes which had been obtained from their own popliteal nodes, and which before injection had been labelled with 3,6,diamino-10 methylacridinium chloride. This is a non-toxic vital nuclear stain, which

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Passage into Bone Marrow

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The Blood Span and the Life Span of the Lymphocyte

In an attempt to throw further light on the fate of the lymphocyte once it enters the blood, numerous experiments have been performed involving a variety of techniques, including the transfusion of lymphocytes with and without some form of labelling, parabiotic preparations, and cross-circulation experiments. In addition, lymphocytes have been labelled—by radio-active isotopes or fluorescent dyes—in the intact organism. Though our main interest is in the lymphocyte, it will be convenient in describing these experiments to refer also to some of the results concerning other leucocytes. It is important, in considering the data about to be presented, to distinguish clearly between the total length of life of the leucocytes (life span), and the length of time they spend in the blood stream (blood span).

kidney. Bone marrow and lymph nodes were unfortunately not examined. They concluded that the failure of the circulating leucocytes to increase after the transfusion was due in the main to their sequestration by the lungs, after both intravenous and intra-arterial injection Leahy, McNickle and Smith (1954) transfused into rabbits labelled leucocytes obtained from peritoneal exudates (produced by sterile saline in animals which had previously been given P^{32}). Eighty-six per cent of the transfused cells were granulocytes, the remainder mainly lymphocytes. They found that within 3–4 minutes after the injection about 72 per cent of the total radioactivity was to be found in the lungs, 3 per cent in the liver, and 1 per cent in the spleen. Twenty-four hours after the injection the mean total activity was 4 per cent in the lungs, 20 per cent in the liver, and 2.8 per cent in the spleen. They inclined to the opinion that the transfused cells were undergoing rapid disintegration

Lawrence, Ervin and Wetrich (1945) made 8 cats leucopenic by irradiation and then established a cross-circulation with normal cats for times ranging from 2 to 22 hours A similar cross-circulation experiment was also performed with one cat which was suffering from infectious feline agranulocytosis. While the cross-circulation was in progress the leucocyte level gradually rose in the leucopenic animal, but fell again when the cross-circulation was discontinued. From the rate of fall (881 white cells per c.mm. per hour) they calculated that the white blood cells of the cat were replaced about one and a half times in 24 hours These experiments are of great interest, but apply unfortunately to all the blood leucocytes, without distinguishing between lymphocytes and other cells.

White (1954) transfused in man leucocytes labelled with atabrine dihydrochloride, which in low concentration appears to have no effect on cell viability. Donor patients were given 50–90 mg of atabrine intravenously, and 500–659 ml of blood were subsequently withdrawn and transfused immediately into the recipient Through a needle in the femoral artery blood samples were obtained from the recipient every 5 min during the first half-hour after transfusion, and every 10 min for the next half-hour. In some cases counts were continued for 7½ hours Atabrine-containing cells were easily recognized by their bright yellow-green fluorescence in ultra-violet light, while untagged cells were invisible in ultra-violet light but were seen by phase contrast illumination

10 patients, with advanced neoplastic disease, received transfusions of atabrine-tagged blood , 5 were auto-transfusions (2 with carcinomatosis, 3 leukaemic) and 5 were hetero-transfusions. In the latter group, 2 cases with non-leukaemic malignancies received leukaemic blood, 2 with carcinomatosis received blood from other patients with carcinomatosis, and 1 leukaemic patient received blood from a case of carcinoma, with granulocytosis following infection. " Following such transfusions, tagged cells

can be readily identified by its fluorescence in near ultraviolet light. Farr found that most of the labelled lymphocytes left the blood within the first 90 minutes, but that in some animals a few lymphocytes remained in the blood for more than 3 hours. A still smaller number of cells could be identified in the blood even after 72 hours; but here the question arises whether they had been in the blood throughout that time, or had been passing in and out of the blood stream. In the main the labelled lymphocytes were found partly in the bone marrow and partly in the various lymphoid tissues. Those in the marrow apparently became transformed into myelocytes, while those in the lymphoid tissues, Farr speculates, may give rise to fresh lymphocytes. Also at 12 hours an occasional lymphocyte could be seen in the lamina propria and submucosa of the intestine, and in connective tissue elsewhere.

Farr's experiments are extremely interesting. If lymphocytes pass from the blood to lymphoid tissues in 2 hours it is possible that they enter the lymphoid tissues not via peripheral lymph, but directly from the blood; this would be the reverse of the process we have previously considered, namely the direct passage of lymphocytes from lymphoid tissues to blood. From a technical point of view Farr's transfusion experiments are extremely satisfactory, for his suspensions consisted of autogenous lymphocytes in sterile pyrogen-free Tyrode solution. Much interest attaches also to the negative finding that he could not detect transfused lymphocytes in the lung. It is unfortunate that no quantitative information is available in his experiments concerning the relative numbers of transfused lymphocytes found in lymphoid tissues and bone marrow respectively.

Boyd *et al.* (1948) fed a rat with glycine labelled with C^{14} and after 24 hours found greater activity in lymphocytes than in granulocytes. This suggests that the turnover of lymphocytes may be appreciably greater than that of granulocytes.

Weisberger *et al* (1951) collected rat lymphocytes from the thoracic duct or a large intestinal tributary. The lymph was collected simultaneously from 5–6 rats for 4 hours and was then centrifuged and the cells washed in saline. Finally they were suspended in 1 ml. of saline, containing 30,000 to 65,000 cells per c.mm. Donor rats were given 0·2 mc. of P^{32} 24 hours before cannulation, and this became incorporated in the DNA of the lymphocyte nuclei, so that the fate of the transfused lymphocytes which had thus been labelled could readily be followed.

They found that the circulating leucocytes fell immediately after the injection of the lymphocyte suspension into the tail vein, and the fall continued for at least 5 hours. When radioactive lymphocytes were employed, about 28 per cent of the radioactivity was found in the lungs, 23 per cent in the liver, 4 per cent in the spleen, and 5 per cent in the

total bone marrow volume, an appreciable part of the total DNAP[32] is in the bone marrow of the recipient animals. However, Fichtelius (loc. cit.) pays little attention to the bone marrow, and appears to incline to the view that lymphocytes are removed from the blood in the spleen and liver. He concludes: "No definite evidence to condemn the hypothesis that the lymphocytes are removed from the blood in the spleen and in the liver has been found in the literature."

Kline and Cliffton (1952) fed P[32] to healthy subjects (6), giving 2·5 mc of P[32] by mouth as inorganic phosphorus. At 24 and 48 hours after administration, and subsequently every 48 hours up to 3 weeks, blood was withdrawn and the DNAP[32] content of the leucocytes was measured. 3–4 days elapse before there is a steep rise in DNAP[32] and the authors assume that this period is required for the formation of the leucocytes in the bone marrow; they did not differentiate between lymphocytes and granulocytes. They estimate that the blood span of the leucocytes after these first 4 days is 8·8 days, hence the life span is 12·8 days. This estimate is far in excess of that given by most other workers.

Fichtelius (1951–1952) also gave P[32] to rabbits, and found a relatively high specific activity in the blood lymphocytes for a few days, rising to a maximum at 4–5 days, then gradually falling off, but attaining a second peak at about 15 days. The appearance of these "second top" leucocytes was also noted in a sheep. This biphasic response is as yet unexplained, but still more curious is the fact (Fichtelius, 1953) that if an animal is bled, "labelled leucocytes can be induced in the blood 4 days after a time when they otherwise do not appear in the blood, and it is probable that this accumulation of labelled cells consists to a large extent of lymphocytes." Fichtelius then endeavoured to trace these lymphocytes when they left the blood, but could not obtain any positive information on this point.

More recently Ottesen (1954) has claimed that by labelling DNA by means of intravenously administered P[32], it can be shown that the blood lymphocytes fall into two distinct groups, of which one has a mean age (blood span) of 3 to 4 days, the other—the majority—of about 100 to 200 days. Ottesen discriminated between lymphocytes and granulocytes, and concluded that as the bulk of the granulocytes enter the blood about 6 days after the administration of P[32] there would be time for the short-lived lymphocytes, which start to enter the blood at 2 days and do so in maximum numbers at 2·5 days, to enter the bone marrow and become transformed into granulocytes. Ottesen (1954) also gives reasons for believing that lymphocytes may spend quite a long time in the lymphoid organs before they enter the blood. It is difficult to reconcile these data with those we have previously cited. It would be very interesting to see Ottesen's experiments repeated in younger—and perfectly normal—subjects. Of the two cases on which Ottesen based his conclusions, one was

were found in the peripheral blood of the recipient for periods ranging from 30–90 min but not thereafter." In non-leukaemic individuals the tagged cells disappeared from the blood more rapidly (often within 30 min) than in leukaemic individuals.

White brings out one point of exceptional interest. Though the numbers of blood leucocytes in the recipient do not show a marked change after the transfusion, their composition alters, since untagged cells become largely replaced by tagged ones. "This would suggest that, at least for the period of the transfusion, there is a rapid emigration of untagged (i e. recipient) cells from the circulating blood, followed by reappearance of the same or other untagged cells as the transfused cells disappear. . . . On this basis, one may further theorize that leucocytes may have many intravascular life spans which may be only a fraction of the life span of the cell."

Lanman, Bierman and Byron (1950) transfused, into non-leukaemic cancer-bearing recipients, blood containing large numbers of mononuclear cells, and found there was only a transient rise in the blood leucocytes.

Osogoe (1950) prepared suspensions of lymphocytes from the mesenteric nodes of young (6 months) rabbits and transfused them to other rabbits. At first most of these lymphocytes were held up in the lungs, but after 24 hours large numbers were found in the liver, and after 48 and 72 hours in the liver and spleen. The cell suspension used contained many large lymphocytes and lymphoblasts It is evident, as Osogoe himself points out, that these experiments do not throw light on the normal fate of the lymphocyte, but rather on the possible histogenesis of lymphoid metaplasia. After 3 weeks the lymphocytic accumulations in the liver had all disappeared

Fichtelius (1953) performed experiments mostly with 3-month-old rats The donor rats were given an injection of P^{32} 24 hours beforehand, and this was taken up in the DNA of the developing lymphocytes A rich suspension of thymocytes in saline was then obtained with a total volume of about 1·0 ml., the cell content being 300,000–340,000 per c mm In most cases cells were transfused between litter mates (syngenesio-transfusion) though heterotransfusion (rabbit to rat) was also employed.

The labelled lymphocytes were identified by extracting the DNAP 24 hours after the transfusion , the number of impulses per mg. $DNAP^{32}$ was then estimated. Fichtelius measured the relative activity of the various organs and found the highest values in the spleen, with bone marrow almost the same, and then in descending order thymus, small intestine, liver, lymph node and lung

The interpretation of these transfusion experiments is difficult for a number of reasons, but it is clear that if one takes into account the

splenic syndrome, the blood from the splenic vein after the injection of epinephrine showed an increase in granulocyte content of 10,000 and 80,000 leucocytes per c.mm. respectively. Ambrus, Ambrus, Johnson and Harrison (1954) report that as far as granulocytes are concerned, the liver and spleen can also sequestrate, though only in small numbers, whereas the capillaries of the limbs do not usually have any effect, except when marked vaso-dilatation or vaso-constriction occur.

Level of Blood Lymphocytes

Lymphocytosis and Lymphocytopenia

The number of lymphocytes in the blood is the result of a balance between incoming and outgoing cells. This will be true whether the equilibrium is relatively static or dynamic, i.e. whether cells merely enter the blood and then when they leave it do so for good, or whether they pass in and out of the blood repeatedly. The level of the blood lymphocytes may therefore be varied through changes in the number either of incoming or outgoing cells, or of both. Lymphocytosis may thus follow (*a*) entry of lymphocytes into the blood in increased numbers, while the number leaving remains unchanged (active lymphocytosis), or (*b*) entry of lymphocytes into the blood in normal numbers, but exit in numbers less than normal, so that there is a retention of lymphocytes in the blood (passive lymphocytosis) Similar considerations apply in the case of lymphopenia.

We may put the position more concisely by saying that, if X = the number of lymphocytes entering, and Y = the number leaving the blood, then normally, over a period:

$$\frac{X}{Y} = 1 \cdot 0 = \text{no change in level}$$

but $$\frac{X^-}{Y}, \frac{X}{Y^+}, \text{ or } \frac{X^-}{Y^+}, \text{ or } \frac{X^+}{Y^{++}} < 1 \cdot 0 = \text{lymphopenia}$$

or $$\frac{X}{Y^-}, \frac{X^+}{Y}, \text{ or } \frac{X^+}{Y^-}, \text{ or } \frac{X^{++}}{Y^+} > 1 \cdot 0 = \text{lymphocytosis}$$

From an examination of the blood lymphocytes alone it is impossible to determine the cause of any change in their number. This can be done, however, by measuring X and Y.

X can be measured in part by counting the lymphocytes passing through the thoracic and right lymph ducts, though as we have already noted, most observers have in fact ignored the right lymph duct and have assumed that X is given to all intents and purposes by the lymphocytes

88 years of age and the other, a 44-year-old woman with a gastric ulcer, was examined by X-rays twice during the course of the experiment. It is just conceivable that this, added to the action of the P^{32}, may have slightly influenced the result. If the findings of Ottesen can be taken at their face value, they would mean that there are two quite distinct varieties of lymphocytes, assuming that one is dealing here with normal cells; alternatively, some of the cells involved may be "stress" lymphocytes

We have thus far been considering mainly the life span of normal lymphocytes; in disease the position may be different. Thus, Osgood *et al* (1952), also using P^{32} for labelling purposes, suggest that in chronic lymphatic leukaemia the life span of the lymphocyte may be of the order of 30 days, whereas in acute lymphatic leukaemia it is less than 7 days They suggest further that the labelling of DNA with P^{32} makes it possible to estimate biochemically the degree of chronicity or acuteness of individual cases of leukaemia.

The Role of the Lungs

It seems abundantly clear that large numbers of leucocytes can disappear with great rapidity from the circulation and in recent years Bierman and his colleagues have, in a series of investigations, drawn attention to the part played in this process by the pulmonary capillaries. Thus Bierman *et al.* (1952) examined in 12 subjects the leucocyte content of blood from peripheral veins and arteries, and also from the right ventricle, pulmonary artery, hepatic vein, left ventricle or aorta. They made repeated leucocyte counts during different stages of respiration, and also during the Valsalva and Mueller manoeuvres. They found that during inspiration the arterial leucocyte count fell, while the venous count rose; the reverse changes occurred on expiration. Fluctuations between 6,000 and 10,000 leucocytes per c.mm. occurred in as short a time as 10 sec. while in 80 sec. the lymphocytes rose from 1,000 to 2,000 per c.mm. These and other changes suggest "a tidal ebb and flow of leucocytes into and from the pulmonary circulation of a significant degree".

The problem has been recently reviewed by Bierman (1955), and it seems clear that as far as this tidal function of the lungs is concerned, it is predominantly the granulocytes which are involved.

The work of Ambrus, Ambrus and Packman (1953), using a Starling heart-lung preparation, suggests that it may be possible to submit to experimental study in animals the mechanism whereby the lungs take up cells from the blood or release them. These workers also agree that it is mainly in the lungs that granulocytes can be removed from the blood in large numbers

But the lungs are not the only organs to be considered. Wright, Doan, Bouroncle and Zollinger (1951) found that, in 2 cases of the hyper-

experiment is the exception rather than the rule; it is not however too infrequent an occurrence, though one which we are as yet unable to explain.

In general, calculations of the I.L.R. on the lines we have just indicated are unsatisfactory mainly because we are unable to measure X with accuracy. (See also Sanders, Florey and Barnes, 1940.) A further complication, even if we could measure it, might be introduced by "stress" reactions (see p 374) However, measurements of thoracic duct lymphocytes may nevertheless be of great value, for they can give a clear indication of the trend in lymphocyte output and tell us whether this is above or below the normal level. Clinically and experimentally a vast number of observations have been made on changes in the blood lymphocytes and it is obvious that their full significance cannot be appreciated until we know *the destination of these cells*. But it may be possible to obtain from the trend of the thoracic duct lymphocyte output an indication as to whether lymphocytosis is due to increased production or diminished elimination of lymphocytes $\left(\frac{X^{+}}{Y} \text{ or } \frac{X}{Y^{-}}\right)$, or whether lymphopenia is due to diminished production or increased elimination $\left(\frac{X^{-}}{Y} \text{ or } \frac{X}{Y^{+}}\right)$ This method of approach has not been utilized sufficiently until now

Lymphocytosis due to Increased Entry of Lymphocytes into Blood

Lymphocytosis due to increased entry of lymphocytes into the blood was first directly observed by Rous (1908*b*), following the administration of pilocarpine. "The intravenous injection of pilocarpine nitrate causes in the dog a rapid and considerable increase in the output of lymphocytes through the thoracic duct The corresponding lymphocytosis induced by the drug in the blood of this animal is not profound, and increased cell-output with the lymph will explain a large part if not all of it . These findings are in accord with the theory that makes mechanical factors responsible for rapidly appearing lymphocytosis" (see Fig 86) Ehrlich and Lazarus (1905) also believed that lymphocytosis in general was of simple mechanical origin. "Accordingly one is obliged to conclude that a lymphocytosis occurs when in response to an increased circulation of lymph in a *greater or less extensive lymphatic region, more elements are* mechanically forced from the lymph-glands. . . . We must, therefore, consider lymphocytosis as the result of a mechanical process" This flushing out of lymphoid tissues is probably responsible for lymphocytosis of rapid onset.

There are, however, several types of more slowly developing lymphocytosis associated with hypertrophy of the lymphoid tissues, in which the

in thoracic duct lymph. If we could assume that such was the case and were to ignore all other lymphocytes entering the blood, the calculation would be simple, for Y could be measured indirectly. By cannulating the thoracic and ligating the right lymphatic duct, we can divert from the blood stream the entering lymphocytes, without preventing the exit of those already in the blood. As a consequence of this procedure, the blood lymphocytes will fall, the total loss (Y) can be calculated from the drop per c.mm. and the blood volume.

The ratio thus calculated of incoming to outgoing lymphocytes we term the Index of Lymphocyte Replacement, or more briefly the I.L.R. In the normal animal with blood lymphocytes maintained at a constant level the true I.L.R. should be 1·0. However, the true I.L.R. cannot at present be calculated and all we can do is to measure the apparent I.L.R., namely the ratio of thoracic duct lymphocytes to those disappearing from the blood.

TABLE 49

Apparent index of lymphocyte replacement in normal dog (ratio of lymphocytes entering blood lymphocytes leaving blood)

Dog no	*Thoracic duct lymphocyte output in millions during the experimental period*	*Blood lymphocyte loss in millions during the experimental period*	*Index of lymphocyte replacement*
1	582	1,175	0·49
2	592	677	0·82
3	2,027	1,753	1·16

Table 49 gives three calculations of the apparent I.L.R. in dogs under chloralose anaesthesia. These calculations are based on cannulation of the thoracic duct without any interference with the right lymph duct. In the anaesthetized (chloralose) dog the I.L.R. measured in this way is usually less than 1·0. This is not surprising, since even if the true I.L.R. were in fact 1·0 the thoracic duct lymphocytes are only a portion of X. Furthermore, in the anaesthetized animal much of the body lymph with its contained lymphocytes is no longer flowing, so that X, even if we could measure it accurately, would probably be diminished. But since Y, the movement of lymphocytes out of the blood, does not depend upon the flow of lymph, it would presumably not be interfered with in the anaesthetized animal, and hence $X < Y$, and the I.L.R. $< 1·0$, so that the blood lymphocytes would fall. This is probably the main reason for the fall in the blood lymphocytes of an anaesthetized animal (Yoffey, 1935).

The last experiment in Table 49 shows an I.L.R. greater than 1·0; in this instance the thoracic duct lymph flow almost trebled towards the middle of the experiment, giving rise to a marked increase in lymphocyte output. Such increase in lymphocyte output during the course of an

experiment is the exception rather than the rule; it is not however too infrequent an occurrence, though one which we are as yet unable to explain

In general, calculations of the I.L.R. on the lines we have just indicated are unsatisfactory mainly because we are unable to measure X with accuracy. (See also Sanders, Florey and Barnes, 1940) A further complication, even if we could measure it, might be introduced by "stress" reactions (see p 374). However, measurements of thoracic duct lymphocytes may nevertheless be of great value, for they can give a clear indication of the trend in lymphocyte output and tell us whether this is above or below the normal level. Clinically and experimentally a vast number of observations have been made on changes in the blood lymphocytes and it is obvious that their full significance cannot be appreciated until we know the destination of these cells. But it may be possible to obtain from the trend of the thoracic duct lymphocyte output an indication as to whether lymphocytosis is due to increased production or diminished elimination of lymphocytes $\left(\frac{X^+}{Y} \text{ or } \frac{X}{Y^-}\right)$, or whether lymphopenia is due to diminished production or increased elimination $\left(\frac{X^-}{Y} \text{ or } \frac{X}{Y^+}\right)$ This method of approach has not been utilized sufficiently until now

Lymphocytosis due to Increased Entry of Lymphocytes into Blood

Lymphocytosis due to increased entry of lymphocytes into the blood was first directly observed by Rous (1908*b*), following the administration of pilocarpine. "The intravenous injection of pilocarpine nitrate causes in the dog a rapid and considerable increase in the output of lymphocytes through the thoracic duct. The corresponding lymphocytosis induced by the drug in the blood of this animal is not profound, and increased cell-output with the lymph will explain a large part if not all of it . . . These findings are in accord with the theory that makes mechanical factors responsible for rapidly appearing lymphocytosis" (see Fig 86) Ehrlich and Lazarus (1905) also believed that lymphocytosis in general was of simple mechanical origin "Accordingly one is obliged to conclude that a lymphocytosis occurs when in response to an increased circulation of lymph in a greater or less extensive lymphatic region, more elements are mechanically forced from the lymph-glands. . . We must, therefore, consider lymphocytosis as the result of a mechanical process" This flushing out of lymphoid tissues is probably responsible for lymphocytosis of rapid onset.

There are, however, several types of more slowly developing lymphocytosis associated with hypertrophy of the lymphoid tissues, in which the

rate of lymphocyte production is apparently stepped up more gradually but remains at a higher level for some time. In none of these cases has the increased lymphocyte output been directly estimated via the thoracic duct, but only inferred from examination of the lymphoid tissues. Thus, Wiseman (1931*a*) found that in the lymphocytosis produced by parentally administered protein the lymph nodes and the spleen showed hyperplastic changes. Nakahara and Murphy (1921) induced lymphocytosis in rats, guinea-pigs, and rabbits by dry heat; in mice, by injecting emulsions of

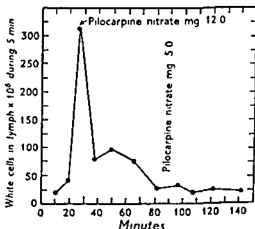


FIG 86 —Chart showing the increased output of thoracic duct lymphocytes in a dog following the administration of pilocarpine nitrate

Ordinates, calculated number of white cells in lymph during 5 minutes, expressed in millions Abscissae, time in minutes

(Redrawn with omission of curves not pertinent to our discussion, from Rous, 1908*b*, chart 5, p 337)

homologous living tissues; and in mice and rabbits, by small doses of X-rays. A lymphocytosis developed which was associated with increased mitotic activity of the germ centres. Von Albertini, Gasser and Wuhrmann (1935–1936) also produced lymphocytosis by means of X-rays but found that the active regeneration was most marked in the diffuse lymphoid tissue of the nodes, not in the germ centres.

It is interesting to note, however, that even under these circumstances there may possibly develop a lymphopenia rather than a lymphocytosis, despite the fact that lymphocyte production is above normal, owing to an excessive demand for lymphocytes elsewhere. This would be a lymphopenia of the $\frac{X^+}{Y^{++}}$ variety, and it is not unlikely that this is the type of lymphopenia which developed in experiments such as those of Lawrence, Dowdy and Valentine (1948), who noted that though regeneration of lymph nodes was complete 20 days after irradiation, there was still lymphopenia

at 25 days. Thoracic duct cannulation in these animals should be able to give definite proof or otherwise of this interpretation.

Some bacteria also stimulate an apparently active lymphocytosis. Reference has already been made to the work of Ehrich (1929*b* and *c*) with staphylococci, in which there was a moderate lymphocytosis with distinct hyperplasia of the lymphoid tissues The effect of *Haemophilus pertussis* is even more striking. It is well known clinically that a marked lymphocytosis may develop during attacks of whooping cough. Tuta (1937) found in rabbits that "an absolute lymphocytosis is produced 24 hours after the intravenous injection into rabbits of living and killed pertussis bacilli", which caused a moderate hyperplasia of the lymphatic tissue in the spleen and mesenteric lymph nodes. In the thoracic duct lymph, the average cell count was 34,000 in normal animals, but no greater than 45,000 per c.mm in the animals injected with pertussis bacilli This suggests a lymphocytosis of the $\frac{X^+}{Y^-}$ type, particularly in view of the hyperplasia of the lymphoid tissues Unfortunately data on the total lymph flow and lymphocyte output are lacking

Harvey (1906–1907) found in rabbits that a lymphocytosis could rapidly be produced by the injection of pilocarpine, muscarine, and barium chloride, the lymphocytosis appearing in as short a time as 10 minutes. Harvey attributed it solely to the contraction of smooth muscle in the spleen and lymphatic nodes and found that it could be prevented "if atropine precedes the incorporation of any drug which stimulates plain muscle tissue, through the motor 'nerve-endings' or a more central part" Meyerhof (1940) reported that an alcoholic extract of the acetone insoluble residue of the mesenteric lymph nodes of the calf caused a lymphocytosis when given subcutaneously to guinea-pigs There are no data upon the effects of such extracts in larger animals or in man

The lymphocytosis, described by many authors, following vigorous exercise of short duration (Ernst and Herxheimer, 1924, Martin, 1932) may be mainly of the mechanical type due to increased lymph flow from the limbs and abdomen. Rous (1908*a*) showed in dogs that "the lymphocyte output may be tripled or quadrupled during a long-continued struggle" But, as noted by Farris (1938*a* and *b* and 1943) and others emotional factors, operating perhaps through an endocrine mechanism, may play a very important part also (For further literature see Farris, 1943, Garrey and Bryan, 1935)

It is perhaps pertinent to refer at this point to the observations of Bierman *et al* (1953) on thoracic duct lymph in man. Leukaemia is generally regarded as being due to overproduction of leucocytes, and in the case of lymphatic leukaemia it would be reasonable to expect some indication, in the thoracic duct lymph, of an increased entry of lympho-

cytes into the blood. Bierman found that in 8 patients with lymphatic leukaemia, the mean lymphocyte content of thoracic duct lymph (taking the highest counts) was 64,600 per c.mm., while the count in peripheral blood at the same time averaged 260,900. This gives a ratio of thoracic duct to blood lymphocytes of 1:4. Bierman and his co-workers interpret their results to mean that leukaemia may not altogether be due to excessive production of lymphocytes and their discharge into the blood. Subsequently Bierman (1955) developed this thesis further. It is, of course, quite possible that leukaemia is due to interference with the passage of lymphocytes out of the blood. But in addition, when one reviews the data of Bierman *et al.* (1953), it is clear that we need more information before we shall be in a position to decide this point. The data so far available certainly suggest that " in some patients with lymphatic leukemia, over-production or excessive delivery of leukocytes is not present at all times " But on the whole, their figures undoubtedly reveal levels of lymphocyte production tending towards the high side, and if there is no change in the number of lymphocytes leaving the blood, leukaemia could be regarded as a sustained and progressive lymphocytosis of the $\frac{X^+}{Y}$ type. A relatively small increase in lymphocyte production would suffice if continued over a long period. This interpretation would be true only on the assumption that both the blood and the life span of the lymphocyte were unchanged, whereas in fact it is quite possible that such is not the case and another variable may thereby be introduced. Thus according to Osgood *et al* (1952) the life span of the lymphocyte may be of the order of 30 days in chronic leukaemia and 7 days or even less in acute leukaemia.

Reinhardt, Ensell and Yoffey (1955) have recently been investigating (guinea-pigs) the output of thoracic duct lymphocytes following splenectomy, an operation which is frequently reported to give rise to chronic lymphocytosis. It was found that 40 days after splenectomy there appeared to be a slightly increased lymphocyte output which was not statistically significant If these results can be substantiated, the lymphocytosis which follows splenectomy would be $\frac{X^+}{Y}$ in type. (See also page 267 for marrow changes after splenectomy)

Lymphopenia due to Diminished Entry of Lymphocytes into Blood

Lymphopenia due to diminished entry of lymphocytes into the blood would perhaps best be exemplified by experiments involving the removal of a sufficient mass of lymphoid tissue. Observations of lymphocyte output in such cases have not been made. In recent years, however, data have become available on lymphocyte output following irradiation (Ross,

Furth and Bigelow, 1952; Brown, Hardenbergh and Tullis, 1950). Thus the latter workers noted that in dogs exposed to 500r total body X-radiation there was a precipitous fall in the cell content of thoracic duct lymph within 4 hours after irradiation, the low level being maintained throughout the observation period (6½ days)

Digestive Leucocytosis

The leucocytosis alleged to follow digestion has an extensive but inconclusive literature, which has been fully reviewed by Arneth and Ostendorf (1923) and by Garry and Bryan (1935). Moleschott (1854) described a two-thirds increase in the blood leucocytes following a meal rich in protein. Since Moleschott's time the occurrence of digestive leucocytosis has been alternately affirmed and denied It seems not unreasonable to suppose that increased peristalsis might be associated with an increased lymph flow from the intestine and a lymphocytosis of the mechanical, flush-out type Goodall, Gulland and Paton (1903–1904) found in dogs that digestive leucocytosis definitely occurred and that it was composite in nature: there was a constant increase in the lymphocytes and a variable increase in the granulocytes. Arneth and Ostendorf (1923) on the other hand deny the existence of a lymphocytosis. In view of these and many other conflicting reports, it is not surprising to find a paper by Videbaek (1946), entitled: "Is 'digestive leukocytosis' a reality?" The reaction, if it occurs, seems to be exceedingly variable and some of the conflicting results may be due to species differences As to the significance of the leucocytosis, we have no information. Paton (1922) suggests that the leucocytes are responsible for the absorption of protein, but there is no convincing evidence that such is actually the case.

Mechanism of Control of Lymphoid Tissue

We do not know how the balance is normally maintained between lymphocytes entering and lymphocytes leaving the blood. In the case of a free cell, such as the circulating lymphocyte, a direct nervous control is impossible, and in recent years haematologists have devoted much attention to hormonal mechanisms More especially the non-specific stress response explains much that was previously obscure, though it has raised additional problems

In one of the earlier reviews of the problem, Hoff (1934) believes that alterations in the acid-base equilibrium are of fundamental importance. Lessening of the alkali reserve is followed by granulocytosis, while an increase stimulates the production of a lymphocytosis. A typical lymphocytosis of this nature is produced by hyperventilation. Similar considerations are applied by Hoff to local inflammatory reactions In acute inflammation the reaction inclines to the acid side with a resultant

outpouring of granulocytes; whereas in chronic inflammation the reaction tends to be neutral or alkaline, and lymphocytes appear on the scene in large numbers The evidence on this point, however, is conflicting Menkin and Warner (1937), examining the exudate which appeared after the intrapleural injection of turpentine in dogs, found that "with the increase in the hydrogen ion concentration to a *p*H below 7·0 polymorphonuclear leukocytes seem unable to survive and the predominating infiltrating cell is the mononuclear phagocyte. A maintenance of an alkaline *p*H . . is accompanied by a preponderance of polymorphonuclear leukocytes. . " Increase in the blood calcium also tends to be associated with granulocytosis

Endocrine Factors

The literature dealing with the influence of hormones upon the leucocytes in general and the lymphocytes in particular has grown considerably during the past decade We propose here to give only a brief outline of the story, as yet incomplete. For a fuller treatment of this problem the reader should consult reviews such as those of Dougherty (1952), Gordon (1954) and Ehrich and Seifter (1953). In discussing the problem it should be emphasized at the outset that it is extremely difficult if not impossible to consider the effects of hormones in isolation, since there is so much interaction between them. It is also difficult to distinguish between a direct hormonal effect and a secondary result of the metabolic changes to which it gives rise

Insulin has been held to play an important rôle. Stockinger and Kober (1931) found that in fasting subjects the subcutaneous injection of 10 units of insulin produced a lymphocytosis which reached its maximum after 2 hours. Such rapid development would seem to indicate a mechanical origin, and Quigley and Barnes (1930) and others have, in fact, shown that insulin causes a hypermotility of the gut, thus suggesting a possible similarity between insulin lymphocytosis and that following pilocarpine. Though for a time the occurrence of insulin lymphocytosis was not universally accepted (e g. Latta and Henderson, 1937, who reviewed the earlier literature), later work has substantiated it (Volk and Lazarus, 1951) and at the same time has shown that glucose exerts an opposite effect in giving rise to lymphopenia, whether administered orally (Freeman and Elmadjan, 1946; Elmadjan, Freeman and Pincus, 1946) or intravenously (Lazarus *et al.*, 1950). The simultaneous administration of insulin and glucose results, according to Volk and Lazarus (1951), in a lesser degree of lymphocytosis than after insulin alone However, as one illustration out of many of the difficulty of analysing hormonal action, Hokfelt (1953) has shown that in the cat insulin stimulates the specific discharge of adrenalin (not *nor*-adrenalin) from the adrenal medulla.

Adrenalin, in turn, can result in the discharge of ACTH from the anterior lobe of the pituitary, and so bring into action the steroid secretion of the adrenal cortex (cf. Zwecker, 1948).

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Jaffe, 1924) points to the occurrence of thymic atrophy after thyroidectomy. Naegeli (1931) states that neither in hyperthyroidism nor after the administration of thyroxin is lymphocytosis a regular occurrence. However, Axelrod and Berman (1951), who give an excellent review of the [illegible]

of toxic hyperthyroidism that this type of reaction occurs, and that in simple hyperthyroidism there is no sign of excessive lymphoid tissue activity Grégoire (1941; 1942*a*, *b* and *c*) has investigated the problem experimentally by administering thyrotropic hormone. He found that the thymus and lymph nodes of rats (or the thymus of chicks) did not enlarge after the administration of thyrotropic hormone, though they did so markedly if the hormone were given after adrenalectomy. Grégoire also induced thymic involution by irradiation and then carefully studied the factors influencing its regeneration.

In recent years the adrenals and to a lesser extent the gonads have received most attention in connection with the control of lymphoid tissue. *From the time when Addison (1855) first noted, in one of the eleven cases* on which he based his description of the disease named after him, a marked increase in the white cells of the blood, evidence has steadily accumulated to indicate a close connexion between lymphoid tissue and the suprarenal cortex In Addison's disease there is usually to be found a marked lymphocytosis, with a condition sometimes resembling status lymphaticus at autopsy (cf Star, 1885) Large numbers of lymphoid follicles may develop in the bone marrow (Hedinger, 1907), though as we have repeatedly emphasized (see also Sundberg, 1955) they are very infrequent in normal marrow.

These and many other clinical observations of this type suggested strongly that the adrenal cortex exerted somehow a depressant effect upon the thymo-lymphatic system, and experimental studies seemed to fit in very well with this idea *Thus* Jaffe (1924) found in rats that the thymus hypertrophied after adrenalectomy, while Marine, Manley and Bauman (1924) obtained the same result in rabbits and in fact noted that if the adrenalectomy had been performed when the thymus was already undergoing involution, regeneration occurred (cf. Simpson, Dennison and Korenchewsky, 1934; Reinhardt and Holmes, 1940).

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lymphopenia and a neutrophilia which they regarded as non-specific, as well as a slight decrease in red cell count and haemoglobin concentration Desoxycorticosterone, on the other hand, was without effect on the blood lymphocytes.

At this point they endeavoured to find some reason for the lymphocytolytic and other degenerative changes in the lymphocytes. They noted that the lymphopenia and lymphoid tissue changes were associated with an increase in the serum proteins, which they then decided were partly antibody globulin, and they concluded therefore that lymphocytes were rich in antibodies. We have dealt elsewhere (p. 270) with some of the basic evidence on antibody formation, and need only cite here some additional work relating to steroid hormones.

Dougherty and White (1944), Kass (1945) and White and Dougherty (1945) also showed that cells—again presumably lymphocytes for the most part—from minced lymph nodes were rich in antibodies. Chase, White and Dougherty (1946) reported that if cortical extract was given together

could produce an increased antibody titre. However, Eisen *et al* (1947) reported that " identical concentrations of serum antibodies and gamma globulin were found in adrenalectomized rats repeatedly injected during immunization with ACE and in similar animals not receiving ACE (extract of suprarenal cortex) " ; a similar finding in cats was reported by Thatcher, Houghton and Zeigler (1948). It is pertinent in this connexion to quote *the experiments of Andreasen et al. (1948), who showed that in the rat* extirpation of 90 per cent of lymphoid tissue was without significant effect on the normal range of serum proteins.

Closely related to the problem of antibody formation is the transfer of sensitivity by cells, lymphocytes or otherwise. (See for example Landsteiner and Chase, 1942 , Chase, 1945 , Haxthausen, 1947 , Stavitsky, 1948 ; Lawrence, 1949 ; Miller and Favour, 1951 ; Wesslén, 1952*b* and *c* and Chase, 1953.) There is evidence that washed cells from sensitized animals, when given to normal animals intravenously, intraperitoneally, or intradermally, can somehow confer sensitivity on the recipient. When the source of the washed cells was peritoneal exudate, buffy coat of peripheral blood, or spleen, " the effectiveness . . . has shown a parallelism with the content of lymphocytes " (Chase, 1953), though exact data are difficult to obtain. Even in the case of lymph nodes, where according to Chase " small lymphocytes are preponderant to the extent of 95 per cent or more ", there is still need for caution in interpreting the results Coons, Leduc and Connolly (1955) point out that " . in a lymph node stimulated by an antigen for the first time, considerably less than 1 per cent of the cells present are engaged in antibody production ".

However, it was not until Selye (1937) described the adaptation syndrome (" alarm reaction ") that really intensive study was made of the relationship between the adrenals and lymphoid tissue. A wide variety of agents functioned as non-specific stress or alarming stimuli, and set in motion changes which resulted in the secretion by the anterior lobe of the pituitary of ACTH, which then entered the blood stream and on reaching the adrenal cortex stimulated the secretion of steroid hormones The range of substances or conditions which can act as alarming stimuli soon became so great, including (Selye, 1940) muscular exercise, exposure to cold, or to the rays of the sun, and even moderate fasting, that in considering the action of almost any substance on the lymphoid tissues one must take into consideration the possibility that the results are a non-specific " stress " response, mediated via the pituitary and adrenals.

One of the striking changes in the alarm reaction is acute involution of the thymus and to a lesser extent of other lymphoid tissues. These changes were analysed in greater detail when supplies of ACTH and purified steroid hormones became more freely available. Thus administration of ACTH was soon shown to result in loss of weight and atrophic changes in lymphoid tissue (e.g. Simpson *et al*, 1943; Sayers, White and Long, 1943; Dougherty and White, 1943; Yoffey and Baxter, 1946, and others). The alterations in the thymus and lymphoid tissues were subsequently studied by Dougherty and White (1944, 1945) and more recently by Baker, Ingle and Li (1951) and by Ringertz, Fagraeus and Berglund (1952) On the whole it is true to say that most observers have confirmed some involutionary effect upon the thymo-lymphatic system of ACTH and 11-oxysteroids, though White and Dougherty (1945) suggested that continued lymphocytolysis might in fact stimulate a strong compensatory lymphocytopoietic reaction.

Yoffey and Baxter (1946) gave daily injections for one month of an aqueous cortical extract to rats and found that it gave rise to a slight but definite hyperplasia of lymphoid tissue. The idea of a reactive hyperplasia was not borne out however by similar experiments with ACTH, associated with depletion of lymph nodes and thymus but with no evidence of increased lymphocyte breakdown Baker, Ingle and Li (1951) treated rats with ACTH for periods up to 21 days and found atrophy most marked in the thymus, but quite evident in lymph nodes also. However, Weir and Heinle (1950) could not bring about lymphoid tissue regression in mice by the administration of cortisone; nevertheless they did not accept Dougherty and White's (1945) suggestion that this was the case because of a secondary hyperplasia.

Dougherty and White (1944) found that in mice, rats and rabbits single injections of pituitary adrenotropic hormone, aqueous or oily cortical extracts, corticosterone, and compound E, produced within a few hours

Yoffey and Baxter (1946), Robertson (1948), and later Baker, Ingle and Li (1951) attributed the depressant effect of ACTH mainly to the inhibition of mitosis. The formation and subsequent detachment of buds from the cytoplasm of lymphocytes had previously been described by Downey and Weidenreich (1912), and there is no doubt that in the examination of normal lymph smears this budding can often be seen, even though its significance is not known (cf Williamson, 1950).

A number of observers have studied the action of steroids upon lymphocytes *in vitro*. Heilman (1945) used hanging drop cultures of lymph node and thought that cortisone slowed cell migration somewhat, while slightly increasing the number of degenerating lymphocytes Robertson (1948) found that over a period of 12 hours aqueous cortical extracts had no obvious effect on lymphocyte numbers, though Hechter and Stone (1948) noted them to be rapidly decreased. Delaunay, [illegible] reported increased disintegration of lymphocytes 21 hours after adding [illegible] hours after cortical acetate. [illegible] ers to have reported rapid [illegible] latively large amounts, and found 90 per cent of the cells dead after 3 hours. Hechter and Johnson (1949) found that adding homogenates of lymphoid tissue augments lymphocytolysis, recalling findings such as those of Herlant (1950) or Ickowicz (1953) in the intact animal. Trowell (1953) found that in the concentrations used cortisone killed lymphocytes, though very much more slowly than *in vivo* His cultures grew on the surface of cotton wool in an atmosphere of pure oxygen.

It is evident that for the most part, steroid substances do not appear to have as marked or speedy an action on lymphocytes *in vitro* as *in vivo*, which suggests the possibility that when administered *in vivo* metabolic derivatives or interaction with other substances may be responsible for the results observed Delaunay, Delaunay and Lebrun (1949) suggest the further possibility that the karyoclasic action of cortical compounds (and adrenaline) was secondary to vascular changes in lymphoid tissue.

Is any purpose served by the breakdown of large numbers of lymphocytes, now that antibody formation can be excluded? White and Dougherty (1947) suggest that lymphocytes on breaking down serve as a source of nitrogen. But from their own data, in which after 48 hours of fasting 9·4 mg of nitrogen were attributable to lymphoid tissue, while 430 mg. came from liver and carcass, it seems that this would be a relatively minor rôle. Ehrich and Seifter (1953) in a stimulating review of the problem suggest that the significance of lymphocytolysis may lie in the

Unless we assume that the development of hypersensitivity and the formation of antibody depend upon quite different cells, it is obviously a possibility to be borne in mind that only a small number of cells need be involved in the process. The experiments of Wesslén (1952*b*), in which the transferring cells were derived from thoracic duct lymph, would appear to implicate the lymphocyte most decisively, though in view of his finding that 5–6 per cent of these cells were large lymphocytes, it is probable that it is these rather than the small lymphocytes which are mainly concerned. But whether this be so or not, all these observations merely serve to emphasize that, however interesting the problem of hypersensitivity may be, it does not appear to throw much light on the rôle of the lymphocyte in normal animals.

Thoracic Duct Output

In cases where the lymphoid tissue showed atrophic changes after ACTH and oxysteroids, the lymphopenia which ensued could be considered to be $\frac{X^{-}}{Y}$ in type, and to investigate this point direct measurements of thoracic duct lymphocyte output were made by several observers. That ACTH diminished the thoracic duct lymphocytes was noted by Reinhardt and Li (1945), Yoffey, Reiss and Baxter (1946), and more recently by Hungerford, Reinhardt and Li (1952). Hungerford and Reinhardt (1948) also noted that adrenalectomy in rats was followed in 2–3 hours by a significant increase in thoracic duct lymphocytes. As against these observations, Valentine, Craddock and Lawrence (1948) were unable to detect any significant change in the thoracic duct lymphocytes of normal and adrenalectomized cats after giving an aqueous adrenal cortical extract —unfortunately they did not try the effect of ACTH.

Hungerford, Reinhardt and Li (1952) found that in rats the administration of ACTH 45 min –2 hours before cannulation significantly diminished the thoracic duct lymphocyte output in normal, though not in adrenalectomized, animals. They also observed that (*a*) hypophysectomy or adrenalectomy raised the lymphocyte output, (*b*) that growth hormone had no effect, and (*c*) that adrenalin diminished the output both in normal and in hypophysectomized rats.

The Action of Steroids on Lymphocytes

When Dougherty and White first noted the action of steroids on lymphoid tissue, they attributed it in the main to a direct action on the lymphocyte and the result was manifested either by the lymphocytes giving off cytoplasmic buds, which they believed to represent the liberation of antibody, or by nuclear damage resulting in pyknosis and cell death.

lymphocytosis 2 hours later, which rose to enormous levels at 8 and 12 hours, and returned to the level found in adrenalectomized non-stressed animals at 24 hours". This they term the "lymphocytotic response" and it would appear to reverse completely the change occurring in the alarm reaction. The lymphocytotic response merits fuller investigation in order to throw light on a mechanism which can so markedly raise the level of the blood lymphocytes, and which may perhaps do so by causing greatly increased lymphopoiesis In this connection it should be noted that Feldman (1951) has claimed that the administration of growth hormone causes lymphoid cells to proliferate and so increases the mass of lymphoid tissue.

Persistence of Lymphoid Tissue

One of the most intriguing features of the thymo-lymphatic involution brought about by ACTH and steroid compounds is that the lymphocytes can never be made to disappear completely. Whether this means that sooner or later a new endocrine equilibrium is established, or that some lymphocytes are more resistant than others, or that there are always a number of lymphocytes arising from primitive precursors, is difficult to say It is hard to explain, on the basis of our present knowledge, why some cases of lymphatic leukaemia should show such marked improvement after ACTH or cortisone, only to relapse again after a varying period. A review such as that by Wintrobe *et al* (1951) brings this point out very forcibly.

In concluding this cursory review of the endocrine control of lymphoid tissue, it should be emphasized that since the first edition of this book appeared there has accumulated an enormous amount of information upon which we have here touched only very briefly and sketchily. No mention has been made of the steroid hormones of the gonads, nor of work such as that of Money, Fager and Rawson (1952), or Santisteban and Dougherty (1954) on the comparative action of different steroid compounds, or for that matter of the effect of administering steroid substances in combination. These are dealt with more fully in the reviews to which we have already referred But enough has been written to make it clear that while we do not fully understand the factors which control the normal development of the lymphoid tissues, important hormonal mechanisms are involved both in quiet conditions, and when the organism is subjected to severe stress.

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delivery of purines and pyrimidines and other split products of nucleoproteins. "As purines have been shown to be powerful stimuli of phosphorylation, or more precisely, since they act as phosphorus transferring enzymes, our results may be interpreted to mean that lymphocytolysis furnishes not only building stones, but also energy for synthetic processes." This is an attractive hypothesis, but one which has yet to be proved; it is a fundamentally different process from the cytoplasmic budding to which we have previously referred; and it does not explain why there is any need for lymphocytes as such ever to pass into the blood stream. A breakdown of lymphocytes could easily occur in localized regions and the soluble disintegration products could then enter the blood stream. There are, then, obvious difficulties about accepting the view that lymphocytes discharge their main function by undergoing lymphocytolysis. The alternative, that "stress" reactions damage lymphoid tissues incidentally, without any useful purpose being served thereby, is also difficult to accept, but taking all things into consideration it seems to present fewer and less serious objections.

Altered Distribution of Lymphocytes

Further, there may be other factors than lymphocytolysis responsible for the lymphopenia. Thus, Yoffey, Metcalf, Herdan and Nairn (1951), employing a quantitative technique for the enumeration of marrow cells, found that in the guinea-pig a single dose of ACTH or of an aqueous cortical extract was followed after 6 hours by an appreciable increase in the marrow lymphocytes. Subsequently Hudson, Herdan and Yoffey (1952) found that after 7 daily injections of ACTH there was an increase in total marrow cellularity, and though there was no significant increase in the marrow lymphocytes there was also no diminution. Yoffey *et al.* (1954) then gave to guinea-pigs repeated injections of compounds E, F and A; again there was no diminution in marrow lymphocytes, but there was a marked increase in the erythroid population. Compound A differed from the other two substances in that it actually gave rise to a lymphocytosis in the peripheral blood. Fruhman and Gordon (1955), applying a somewhat different quantitative technique, obtained a similar type of result in the bone marrow of the rat after the administration of corticosterone.

A Lymphopoietic Component of Stress

Thus far, in discussing the relation between stress and lymphoid tissue, we have been considering it in terms of ACTH and the secretion of the adrenal cortex. A further complication however has been introduced by Dougherty and Kumagai (1953), who found that stress stimuli administered to mice 2 hours after adrenalectomy gave rise to "a significant

lymphocytosis 2 hours later, which rose to enormous levels at 8 and 12 hours, and returned to the level found in adrenalectomized non-stressed animals at 24 hours". This they term the "lymphocytotic response" and it would appear to reverse completely the change occurring in the alarm reaction. The lymphocytotic response merits fuller investigation in order to throw light on a mechanism which can so markedly raise the level of the blood lymphocytes, and which may perhaps do so by causing greatly increased lymphopoiesis. In this connection it should be noted that Feldman (1951) has claimed that the administration of growth hormone causes lymphoid cells to proliferate and so increases the mass of lymphoid tissue

Persistence of Lymphoid Tissue

One of the most intriguing features of the thymo-lymphatic involution brought about by ACTH and steroid compounds is that the lymphocytes can never be made to disappear completely. Whether this means that sooner or later a new endocrine equilibrium is established, or that some lymphocytes are more resistant than others, or that there are always a number of lymphocytes arising from primitive precursors, is difficult to say It is hard to explain, on the basis of our present knowledge, why some cases of lymphatic leukaemia should show such marked improvement after ACTH or cortisone, only to relapse again after a varying period. A review such as that by Wintrobe *et al* (1951) brings this point out very forcibly.

In concluding this cursory review of the endocrine control of lymphoid tissue, it should be emphasized that since the first edition of this book appeared there has accumulated an enormous amount of information upon which we have here touched only very briefly and sketchily No mention has been made of the steroid hormones of the gonads, nor of work such as that of Money, Fager and Rawson (1952), or Santisteban and Dougherty (1954) on the comparative action of different steroid compounds, or for that matter of the effect of administering steroid substances in combination These are dealt with more fully in the reviews to which we have already referred. But enough has been written to make it clear that while we do not fully understand the factors which control the normal development of the lymphoid tissues, important hormonal mechanisms are involved both in quiet conditions, and when the organism is subjected to severe stress.

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CHAPTER 7

THE LYMPHOCYTE

General Problem

The lymphocyte is a somewhat inconspicuous cell, with no particularly striking functional or morphological characteristics. The cytoplasm appears to be singularly undifferentiated and unspecialized when it is compared with the other white cells of the blood. The nucleus does not show any obvious lobulation, though often, when it appears at first sight to be perfectly rounded, on careful examination it may be seen to possess one or more indentations and occasionally a deep and narrow fissure, which makes it the shape of a horseshoe with the limbs closely pressed together (cf Maximow, 1932). Comparing it with other cells, one thinks of the lymphocyte in negative terms, defining it rather by the absence of characteristics which other white cells possess than by positive attributes of its own Yet it has been for many years, and still is, the cell around which a violent haematological controversy has been waged Lewis (1933) rightly observes that " its fate . is the subject of religious beliefs ".

A careful study of the relation between the various blood and connective tissue cells only became possible with the introduction of the differential staining of fixed smears by Ehrlich (1879) Since then the main lines of the controversy have remained unchanged in principle, though with modifications as new techniques—such as tissue culture and vital and supravital staining—were introduced The views of Ehrlich, as the founder of modern haematology, carried especial weight. It was Ehrlich who first advanced the idea that the lymphocytes are a cell group *sui generis*, completely unconnected with any other cells of the blood, bone marrow, or connective tissues He based this view on the absence of granules, associated with the inability to move On account of the absence of motility, so Ehrlich believed, the lymphocytes were unable to migrate from the blood vessels in inflammatory processes The fact that they were, nevertheless, found in large numbers outside the vessels in certain inflammatory reactions " by no means proves that they wandered out from the vessels " (Ehrlich and Lazarus, 1905)

Although it was subsequently shown that the lymphocyte possessed the power of movement and of migrating through vessel walls, Ehrlich's negative approach to the lymphocyte problem still persisted. It was further developed by Schridde (1913) and Naegeli (1931), though the grounds on which this approach was based had to be shifted; for, in the

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the pseudopod increases in length and breadth the groove usually becomes more marked" The part of the lymphocyte behind the constriction ring passes forward through the ring, which remains fixed in relation to points outside the moving cell. After the nucleus has gone through, the ring contracts down, giving *the cytoplasm behind it the appearance of a tail.* The lymphocyte squeezes through the ring much as one would imagine it would squeeze through a small opening between endothelial cells. In our migrating lymphocytes the groove is not produced by an external factor, for they migrate in this way when they are on the coverglass in a fluid medium. When they are in a thick plasma clot they do sometimes squeeze through small meshes of the fibrin and it may be that since lymphocytes have been so accustomed, for thousands of generations, to squeezing through tight places they go on acting this way when there are no small holes to go through. In other words, this groove formation is an inherited feature."

McCutcheon (1924), examining small lymphocytes of human blood *in vitro*, found that 54 per cent of the cells were obviously moving at the end of the first hour, and all by the ninth hour. The speed increased from 4 μ per minute in the first hour to 15 μ per minute in the ninth, with a maximum of 40 μ. Neutrophilic granulocytes moved at a slightly faster rate, attaining a maximum speed (34 μ per minute) by the third hour and all exhibiting movement even during the first hour Abramson (1925), investigating lymphocytes in serum, found that with the passage of an electric current all the lymphocytes started to move in the same direction—i e in a straight line toward the anode—at speeds varying from 12 – to 17 – μ per minute. We do not know whether electrical changes ever determine lymphocyte migration in the tissues Abramson makes the suggestion that the current of injury, which is of the *same order as the* electromotive force used in his experiments, may stimulate such migration. He further noted that if the lymphocytes were kept on ice for 30 hours and then examined on the warm stage their motility was unimpaired

Using mixtures of lymphocytes and of *polymorphonuclear leucocytes*, Abramson (1927) noted that "the lymphocytes overtake the leucocytes " Although one may occasionally observe "a leucocyte exhibiting the unusual property of migrating faster than a lymphocyte. This, *however, is rare*"

It has been claimed that the typical lymphocytic movement may be of use in identifying cells whose classification proves difficult. Thus Rich (1936) investigating the splenic enlargement in rabbits following the *parenteral injection of foreign protein, found numerous* large cells with vesicular nuclei and abundant basophilic cytoplasm These could have been interpreted as either macrophages or myeloblasts, but motion picture studies showed that their mode of progression was typically lymphocytic,

meantime, blood formation had been studied in greater detail and it had become clear that both the granulocytes and the erythrocytes were derived from parent cells with rounded nuclei and basophilic, non-granular cytoplasm The line of separation of these " indifferent " cells from the lymphocytes was not distinct and Pappenheim (1899), Wolff (1902), Maximow (1902), and others began to suggest that no real difference existed between lymphocytes and myeloid and connective tissue cells—a view from which Ehrlich strongly dissented. " . . . a peculiar retrograde movement in the theory of leukocytes has been put on foot. Although after Virchow's description of the lymphocytes every effort was made to separate the different varieties of white blood corpuscles from one another, and refer them when possible to their different sources, now every effort is directed toward placing all the leukocytes under one roof on the theory that the different forms are only different stages of development of the same cell " (Ehrlich and Lazarus, 1905) This is the essence of the problem which still confronts us ; whether we can place all the leucocytes " under one roof ", or not. And although much new evidence has accumulated since Ehrlich's time, we are still not in a position to answer the question with absolute finality.

If the lymphocyte is not the precursor of other cells, but has its own independent life cycle, it ought to be possible sooner or later to demonstrate some special lymphocytic function. The fact that no such function has been satisfactorily demonstrated, in spite of numerous attempts, is in itself significant Before considering the question of the relation of the lymphocyte to other cells, however, let us review its known biological properties.

Biological Properties of the Lymphocyte

Movement

Lymphocytes are not normally capable of phagocytosis, though Downey (1916–1917 ; 1917), and Lang (1926 , 1938) have noted that under certain circumstances they may ingest dyes and India ink, at times even intravascularly

Lymphocytes of all sizes are capable of movement. Lewis (1931, 1933) has studied the motility of lymphocytes in detail. At rest, the lymphocyte is spherical, but " pseudopodia are sometimes thrust out and retracted without producing more than a slight wabbling motion of the whole cell. When, however, the rest period is over and the lymphocyte begins to migrate, a single pseudopodium flows out from a relatively small area. As the pseudopodium enlarges, a groove develops at its base where it joins the body of the cell This groove or constriction ring corresponds to the rim about the softened area which gives rise to the pseudopod As

can barely be seen, it seems unlikely that it would possess an extensive secretory function. If such a function exists it must be almost entirely nuclear. Nor does it seem reasonable—when we consider the problem of fat or of protein transport by lymphocytes—to suppose that the small lymphocyte with its scanty cytoplasm should be able to transport these materials to any considerable extent. Even the larger lymphocytes, with rather more cytoplasm, rarely contain fat in stainable form (cf. Bloom and Wislocki, 1950) though there is some evidence to suggest that they *may contain proteolytic enzymes. In this connection it is pertinent to* bear in mind that most of the lymphocytes entering or circulating in the blood possess but little cytoplasm.

Proteolytic Enzymes

Hedin and Rowland (1901) showed that lymph nodes underwent autolysis more rapidly in an acid than in alkaline medium. Opie (1906) extended this observation to scrapings from the mesenteric node of a dog and concluded, "The name *lympho-protease* may be given to that enzyme which is contained in the large mononuclear phagocytes since these cells have their origin in lymphoid tissue". It is not clear from Opie's experiments whether lymphocytes themselves contain protease, for the scrapings which he used would contain many of the reticular cells of the sinuses and even some granulocytes. Longcope and Donhauser (1908) wrote of a case of acute large-celled lymphatic leukaemia, "The leucocytes of the circulating blood and of the enlarged lymph nodes . . contain proteolytic enzymes that act qualitatively in much the same way as the leucocytes of pus and as the white corpuscles of the blood in myelogenous leukaemia". They confirmed the earlier views as to the proteolysis being most marked in an acid medium. "The enzyme of the large lymphocytes seems to act better than the enzymes of the myelocytes and polymorphonuclear leucocytes when the reaction of the solution is acid". In the small lymphocytes, however, no proteolysis could be demonstrated, whatever the reaction of the medium, and Longcope and Donhauser conclude that "the large cells of the so-called acute lymphatic leukaemia are not true lymphocytes, but are nearly related to the granular myelocytes and should probably be considered as the forerunners to these cells".

According to Morris and Boggs (1911), on the other hand, a protease could be demonstrated in the small lymphocytes of chronic lymphatic leukemia, even in neutral media, and these authors therefore came to *precisely the opposite conclusion, namely, that* "*biological differences* between myeloid cells and the lymphocytes of chronic lymphoid leukaemia have not been demonstrated in neutral media".

Rona and Kleinmann (1931) investigated the proteolytic properties of extracts of lymph nodes and of spleen. They found, using a variety

and they were to be regarded therefore, as having arisen from the lymphocytes of the malpighian follicles. Similarly, Rich, Wintrobe and Lewis (1939) thought it possible to distinguish lymphoblasts from myeloblasts by their special mode of locomotion.

However, the studies of De Bruyn (1945) cast doubt on the validity of this criterion In cultures of rabbit lymph nodes he described a gradual hypertrophy of lymphocytes and their transformation into macrophages, the change from one cell type to the other being accompanied by " forms of movement intermediate between the migration of the typical lymphocyte and that of the typical macrophage ".

The movement of the lymphocyte is characteristically biphasic. During the locomotive phase the cell becomes polarized, as already noted. During the non-locomotive phase the cell becomes depolarized and returns to its resting condition. This is in accord with the description given by Lewis (1931, 1933) and also by Ebert, Sanders and Florey (1940) De Bruyn also confirmed that during the depolarized phase the lymphocyte is not really at rest, but is continually putting forth and retracting small pseudopodia, which are not very obvious simply because the cell possesses so little cytoplasm

The motion of the macrophage, on the other hand, is continually depolarized and shows the typical undulating movement of its cytoplasm all around its border De Bruyn then observed (*a*) that the lymphocytes in his cultures underwent gradual hypertrophy, ultimately becoming macrophages, and that (*b*) as they did so, the depolarized phase of the lymphocytes became more and more like that of the macrophages, while at the same time the locomotion phase became less frequent until finally it disappeared The occasional reappearance of the typical lymphocyte movement in a cell which might otherwise have been looked upon as a monocyte was regarded as evidence against its being a monocyte undergoing enlargement.

The motion of lymphocytes and thymocytes is identical (Murray, 1947, and many others). There is as yet no known chemotactic stimulus for the lymphocyte (McCutcheon, 1955), though Pulvertaft and Jayne (1953) thought they were attracted towards malignant cells.

Secretion and Absorption

Numerous observers have attributed to the lymphocyte chemical functions in the form either of secretion or of absorption. A comparison with other secretory cells brings out certain general features of interest. Whatever the part played by the nucleus, the secretory activities of a cell usually find morphological expression in a well-developed cytoplasm, which frequently acquires specific granules. In the case of the small lymphocyte, where the cytoplasm is merely a narrow rim, which at times

in a review published in 1921. Fiessinger (1923) also believed in the production of lipase by lymphocytes. It is probable, however, that in some of his experiments Bergel was not dealing exclusively with lymphocytes (cf. Aschoff and Kamiya, 1922).

Numerous observers have noted that lymphocytes do not contain histologically demonstrable lipoids. Sehrt (1927) was unable to stain lipoid material in lymphocytes with either Sudan III or Nile blue sulphate. Baillif and Kimbrough (1947) were equally unsuccessful with Sudan black B, as were Bloom and Wislocki (1950) with both Sudan black and the acid haematein reaction.

As to phosphatases, Wachstein (1946) was unable to demonstrate the occurrence of alkaline phosphatase in the leucocytes of the blood, though Haight and Rossiter (1950) were able to show that lymphocytes contained acid phosphatase (see also Rabinovitch and Andreacci, 1949) Wislocki and Dempsey (1946) note the occurrence of alkaline phosphatase in lymphatic nodules of spleen, lymph glands and bone marrow. The reaction is marked in the peripheral part of the nodules and least evident in the cells of the central portion

Glycogen Content

The question of glycolytic enzymes, which we shall next discuss, involves the possibility of glycogen being present in the cells Wagner (1946, 1947), working with the whole blood and leucocytes of buffy coat, was unable to detect glycogen in lymphocytes by macrochemical methods. Wachstein (1949), using the periodic acid-Schiff technique, was unable to demonstrate more than a trace of glycogen in lymphocytes of human blood, thus differing from Gibb and Stowell (1949), who found that the Hotchkiss method "revealed glycogen in nearly all lymphocytes" Reference has previously been made to the observations of Smith and Thomas (1950) on the presence of glycogen in the cortical cells of the mouse thymus for about 14 days after birth.

Metabolism

Soler (1924) thought that lymphocytes were capable of storing carbohydrates Several workers have shown that these cells can effect both aerobic and anaerobic glycolysis Barron and Harrop (1929) studied small lymphocytes from the blood of patients with chronic lymphatic leukaemia and also from dog's thoracic duct lymph. Comparing lymphocytes with granulocytes, these investigators found there was no marked difference in oxygen consumption between the two cell types. When calculating oxygen consumption per million cells per cubic millimetre, per unit of time (one hour), they found that oxygen consumption tended to be in inverse proportion to the cell concentration (see Table 50). " . . . the

of substrates, that lymph node extracts contained two ferments: one a cathepsin, most active at pH 4 to 5; and the other a trypsin, present in much smaller amount and most active at pH 7 to 8. The source of the trypsin is open to doubt; it may have been derived from granulocytes or macrophages. But the cathepsin would seem to correspond to the proteolytic enzyme which earlier workers found in pure lymphocyte suspensions. In the case of lymph node and spleen extracts, however, it is manifestly impossible to decide whether this cathepsin is derived from small or from large lymphocytes.

Barnes (1940) made a careful study of the enzyme content of lymphocytes obtained from the thoracic duct lymph of cats and rabbits He found that in both species the lymphocytes contained cathepsin, nuclease, lipase and lysozyme, while in the rabbit they also contained amylase and were especially rich in adenosinase. Wagner and Ehrich (1950), investigating the enzyme content of the rabbit's popliteal node, were impressed by a marked increase in the adenosinase content of this node after the injection of vaccine into the pad of the foot But in view of the marked species differences it is evidently difficult to generalize about the function of lymphoid tissues on the basis of these rabbit experiments. Fleisher (1955), investigating the dipeptidase and tripeptidase content of leucocytes, found that in regard to the glycyl-glycine splitting dipeptidase, " Lymphocytes appear to be significantly more active than polymorphonuclear leukocytes."

Faerber (1939) could not find cytochrome in lymphocytes But Hoffman, Rottino and Stern (1951) were able to show that leucocytes both from normal and diseased nodes contained small but definite amounts of cytochrome oxidase, visualized by means of the G Nadi reaction However, because of the low level of cytochrome activity they concluded either that lymphocytes have a low energy requirement, or that they possess sources of energy other than carbohydrates, e.g. energy-rich phosphate bonds in the form of adenosine triphosphate Ehrich and Seifter (1953), in the light of Wagner and Ehrich's (1950) experiments to which we have just referred, suggest that the purines which are formed on lymphocyte breakdown act as phosphorus-transferring enzymes, so that lymphocytolysis furnishes not only building stones, but also energy for synthetic processes. Such an interpretation may be feasible in the rabbit whose lymphocytes are rich in adenosinase, but is not so readily tenable in the cat whose lymphocytes contain no adenosinase (Barnes, 1940), the position in other animals has yet to be determined.

Lipolytic and other Enzymes

Bergel has been the most consistent advocate of the view that the lymphocytes secrete a lipolytic enzyme; a summary of his work is given

with Peschel (1930) that the metabolism of granulocytes resembles that of malignant tissues, while that of lymphocytes is similar to normal adult tissues

With slices of lymph nodes, Victor and Potter (1934) found in the normal mouse an oxygen consumption of 5·5 c mm./mg. dry weight per hour; and with single nodes Victor (1935) reported an oxygen consumption of 4·6 c.mm /mg. dry weight per hour.

Carriage of Virus

The experiments of Yoffey and Sullivan (1939) indicate that lymphocytes play an important rôle in the dissemination of vaccinia virus from a focus of infection Even if this were true for vaccinia virus only, the frequency with which this virus is used in Jennerian vaccination makes the fact significant. After nasal instillation in the rabbit, vaccinia virus was found in the cervical lymph at all times from 12 hours to 7 days afterwards The lymph containing the virus had traversed one or more nodes and was passing without further nodal interruption to the blood When such lymph was centrifuged for 15 minutes at 2,000 r p m., virus was found only in the sediment. Centrifuging at this speed cannot throw down free virus The virus therefore must have come down with the sediment, which consisted of a few red cells and large numbers of white cells, shown by smears to be almost 100 per cent lymphocytes The earlier work of Todd (1928), Smith (1929) and Douglas and Smith (1930) would appear to exclude the red cell as a virus carrier. Later workers (e g Andrewes, 1944) generally inclined to the view that " virus is adsorbed to erythrocytes, but after a period of time comes off again " The electron micrographs of Reagen *et al* (1953) suggest that it may be possible to obtain visual confirmation of this. But in view of the comparatively small number of red cells in lymph it would seem more than likely that in the cervical lymph sediment it is the lymphocytes which carry virus Unpublished experiments of ours indicate that fixation of virus by lymphocytes occurs *in vitro*, thus extending to the lymphocyte a property which has already been demonstrated by Douglas and Smith (1930) and by Sabin (1935) for the blood leucocytes as a whole, by Rous, McMaster and Hudack (1935) for mixed cell suspensions from rabbit embryos; and by Beard and Rous (1938) for isolated Kupffer cells

Does fixation by the lymphocyte have any effect on the virus? We do not know, but several workers have studied mixtures of vaccinia virus with other cells and their observations are interesting from the point of view of the mechanism of immunity to viruses. (See Smith, 1929; Fairbrother, 1933; Sabin, 1935, Rous, McMaster and Hudack, 1935; Beard and Rous, 1938) Sabin (1935) showed that (*a*) " fresh normal rabbit serum and leucocytes are not viricidal for vaccinia ", (*b*) that " in

optimum concentration for maximal respiration and maximal glycolysis of both granulocytes and lymphocytes is approximately 10,000 cells per c.mm." This may possibly be significant, since it is the order of concentration in which leucocytes are normally present in the blood—and also, very frequently, lymphocytes in thoracic duct lymph.

When there is overcrowding, the lymphocytes show a greater diminution of respiratory power than do the granulocytes This suggests that the closely packed masses of lymphocytes in lymphoid tissue may have a relatively low oxygen consumption; whereas, when the lymphocytes become detached and enter the blood, their oxygen consumption rapidly rises.

If experiments are compared in which the cell concentrations are approximately the same, Barron and Harrop (1929) found that the aerobic glycolysis of granulocytes is about five times that of lymphocytes Peschel (1930) examining the lymphocytes from four cases of lymphatic leukaemia could find no aerobic glycolysis Anaerobic glycolysis also is much greater for granulocytes than lymphocytes. In this respect the granulocytes resemble tumor and retinal cells, whereas the lymphocytes resemble the

TABLE 50

Oxygen consumption of lymphocytes, with effects of overcrowding

Source of material	*No of cells per c mm*	*O_2 consumption per hour c mm*	*K**	*Lymphocytes* %
1. Chronic lymphatic leukemia	150,000	60 0	0 40	95
2 ,, ,, ,,	52,800	56 2	1 06	90
3 ,, ,, ,,	320,000	54·0	0 017	97
1 Dog lymph lymphocytes	10,000	25	2 5	98
2 ,, ,, ,,	8,000	23	2 87	92
3. ,, ,, ,,	6,000	12 5	2 08	93

$$* K = \frac{\text{c mm } O_2 \text{ consumed}}{\text{cell concentration (millions)} \times \text{time (hours)}}$$

From Barron and Harrop (1929)

cells of normal tissues. Barron and Harrop also found that the metabolism of the lymphocytes was adversely affected by prolonged anaesthesia, centrifuging and the use of sodium citrate as an anticoagulant. One or more of these factors may come into play when obtaining lymphocytes for purposes of tissue culture. The possibility that the cells used are in bad shape to begin with may contribute to some of the negative results of culture experiments. Soffer and Wintrobe (1932) noted that the glycolytic power of granulocytes was about twice that of lymphocytes, while their oxygen consumption was also a little greater. They agree

becomes explicable by blood transmission. This does not exclude the possibility of other modes of spread, but may render them rather more difficult to prove and also less necessary to assume. (3) A striking pathological change in many virus diseases is the perivascular accumulation of lymphocytes. This is usually interpreted as reaction to the virus. It is tempting to speculate that it may—at any rate in part—be cause rather than effect, and that virus is first brought to the affected region by a few scattered cells, though the main accumulation of lymphocytes subsequently is a reaction to the virus.

The fixation of virus by the lymphocyte makes it easy to understand why the lymph nodes offer so little obstruction to the passage of virus. From the work of Drinker, Wislocki and Field (1933) we know that particles reaching a node first pass through the cortical sinus; and that since the wall of the sinus is incomplete where it borders the nodules of lymphoid tissue, the particles as they pass through the sinus come into close contact with the lymphocytes, without any intervening endothelium. Particles of India ink or graphite perfused through the node may even be found to have penetrated in between the densely packed lymphocytes (see Fig 78) Hence virus entering the cortical sinus would have every opportunity of coming into intimate contact with the lymphocytes and of becoming fixed by them Since these cells are constantly leaving the nodes in large numbers, it follows that unless the node becomes completely disorganized virus also must be leaving it via the efferent lymph.

To what extent does the mechanism of spread which has been demonstrated for vaccinia virus hold good for other viruses? In the case of vaccinia virus it appears to depend on fixation of the virus by lymphocytes, and since other viruses are also cytotropic, it is conceivable that they also may spread in the same way. It is a necessary corollary to this mode of spread that virus should be found in the blood-stream, this is actually the case in those virus diseases which have insect vectors and in many, if not all, other virus diseases

The Virus of Poliomyelitis

The problem of virus spread assumes a special interest in connection with the so-called neurotropic viruses, especially that of poliomyelitis. Burrows (1931), investigating the pathology of the disease in 50 autopsies, concludes that the nervous involvement is only a rare complication in a widespread infectious disease. " . . it seems that the disease must enter by way of the gastro-intestinal lymphatics (Peyer's patches and the solitary follicles). Symptoms in the latter region are always present. . . From this portal of entry the disease spreads to contiguous lymphatic tissues in the body. In the mild cases, immunity and recovery result after a limited amount of this tissue is involved. In the less resistant cases,

the presence of normal serum leucocytes 'fix' the virus, but become and remain highly infectious thereby". He also noted that even in the presence of immune serum, virus fixed by leucocytes was not destroyed. Florman and Enders (1942) showed that in roller tube cultures of rabbit white blood cells, to which vaccinia virus was added, the virus multiplied appreciably, even when they used leucocytes from immune animals. They therefore conclude that "the mononuclear blood-cell affords a site in which the virus of vaccinia may multiply", and they think it "probable, since the virus exerts no apparent injurious effect on mononuclear blood-cells in culture, that the two daughter cells of an infected cell which *underwent division would each retain a share of the virus, and in this* way theoretically the virus might be preserved indefinitely provided further multiplication of it took place in the newly formed cells".

The problem of the distribution of the virus after it has entered the blood is bound up with the problem of what happens to the lymphocytes. It has been shown (Yoffey and Drinker, 1939*a*) that some of the blood lymphocytes enter the lymph stream, passing en route through the connective tissues. This means, in the first place, that in vaccinia there may be a diffuse vaccinial involvement of all the connective tissues. It also means that we have here a ready means of infecting all the lymph nodes in the body, since lymphocytes which have entered peripheral lymph must pass through one or more nodes before returning to the blood. Following this line of reasoning, Yoffey and Sullivan (1939) examined popliteal nodes for the presence of virus after nasal instillation and actually found virus in two experiments out of six. This does not prove, of course, that virus reached the nodes by way of the lymph stream. It may equally well have reached them directly from the blood But, whatever the route, the important fact is that virus may be found in lymph nodes remote from the drainage area of the primary focus of infection.

Once virus has reached a node, it can multiply there (McMaster and Kidd, 1937) and the node thus becomes a new centre for the dissemination of virus, which passes out with the lymphocytes into the efferent lymph, ultimately reaching the blood via the thoracic or right lymphatic ducts. It is possible that this is the true explanation of the almost constant presence of virus in the thoracic duct lymph in the experiments cited in Chapter 5. A similar chain of events very probably takes place in clinical (Jennerian) vaccination

That a virus can be fixed by the lymphocytes may be significant from several points of view: (1) if the virus is intracellular, it is protected while passing through lymph or blood against any neutralizing principle which these fluids may contain (2) Since the lymphocyte is able to migrate through the walls of the capillaries, virus can leave the blood-stream in all parts of the body. The finding of virus in any given tissue, therefore,

becomes explicable by blood transmission. This does not exclude the possibility of other modes of spread, but may render them rather more difficult to prove and also less necessary to assume. (3) A striking pathological change in many virus diseases is the perivascular accumulation of lymphocytes. This is usually interpreted as reaction to the virus. It is tempting to speculate that it may—at any rate in part—be cause rather than effect, and that virus is first brought to the affected region by a few scattered cells, though the main accumulation of lymphocytes subsequently is a reaction to the virus.

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wider extension results, even to the involvement of the lymphoid structures of the central nervous system." Burrows finally suggests that the name "infantile paralysis" is misleading, since it describes what is only an *occasional complication*, and that "acute lymphatic hyperplasia" is a more accurate description in the majority of cases (cf. also Sommers, Wilson and Hartmann, 1951) Burrows' surmise about entry via the intestinal lymphoid nodules receives confirmation in the frequency with which poliomyelitis virus has been found in the ileum (Sabin and Ward, 1941, Wenner and Paul, 1947)

However, the concept of a neurotropic spread was aided by the fact that, apart from the alimentary canal, virus was only infrequently found in association with lymphoid tissue, and the strains of virus which were more commonly used for experimental work in animals showed a very marked neurotropism (Fairbrother and Hurst, 1930; Faber, 1933; Flexner, 1936). Enders, Weller and Robbins (1949) demonstrated by tissue culture methods that freshly isolated virus could grow readily outside the central nervous system and possibly in complete independence of nervous tissue. Subsequently Wenner and Rabe (1951)—who summarize earlier reports of the occasional finding of virus in lymphoid tissue—were able to identify poliomyelitis virus in the lymph nodes of 6 out of 9 fatal cases of poliomyelitis.

It is clear, of course, as has already been noted, that the lymphocytic dissemination of virus involves as an essential corollary the presence of virus in the blood It is interesting therefore to observe that the occurrence of viremia in cases of poliomyelitis has latterly been demonstrated both in animal experiments, and in man (see Bodian, 1952; Koprowski, Norton and McDermott, 1947, Horstmann, 1952; Verlinde and Beem, 1952) The viremia, as one might expect, always precedes the invasion of the central nervous system.

Though the actual presence of poliomyelitis virus in lymphocytes has not yet been demonstrated, it seems not unreasonable to suppose that a freshly isolated, pantropic strain of virus could readily flourish in lymphoid tissue and lymphocytes Conversely, our own failure to find poliomyelitis virus in lymph or lymphoid tissue (Yoffey and Drinker, 1939*b*), as well as the negative results of many other earlier workers, may well have been due, *inter alia*, to the employment of strains of virus which had been rendered much more specifically neurotropic by repeated intracerebral injection. Be that as it may, we were never able to detect poliomyelitis virus in lymph. Cervical lymph was obtained in a number of experiments from monkeys infected either by intranasal instillation or by the intracerebral injection of virus, and at all stages of the disease. In 3 experiments, specimens of thoracic duct lymph were obtained from paralysed monkeys But not once could we demonstrate—by intracerebral in-

oculation into normal monkeys—the presence of virus in lymph from infected animals. In the light of recent work, these experiments need to be repeated, with freshly isolated virus, in an animal such as the chimpanzee.

Lymphoid Tissue in other Virus Diseases

There can be little doubt that there is diffuse involvement of lymphoid tissue in many virus diseases, and at times it may give rise to pathological changes which are readily recognized.

In infectious mononucleosis Downey and Stasney (1935–1936) made a careful study of the lymph nodes at different stages of the disease (see also Moeschlin, 1940–1941). They concluded that "The histological and cytological features described, together with the blood picture, indicate a reaction that is more or less leukemoid in nature to an infective, toxic, lymphotropic agent. The histological picture points towards location of this agent within the lymph nodes . . ." They further emphasize the passage of abnormal lymphocytes into the blood; the presence of such cells has been stressed latterly by Fisher, Ranier and Crawford (1950) and by Litwins and Leibowitz (1951) in a number of virus diseases—chicken pox, influenza, virus hepatitis, herpes zoster, herpes simplex, roseola infantum, rubella, influenza type B, undulant fever, and rickettsial pox, as well as in infectious mononucleosis. Litwins and Leibowitz actually suggest the name of "virocytes" for these abnormal lymphocytes occurring in the blood in virus diseases. They go so far as to say that if virocytes are found in apparently normal individuals, or in individuals suffering from other than virus diseases, the "utmost caution should be exercised to determine that an intercurrent infection of virus origin has not intervened, either naturally, or by artificial introduction by the parenteral route." They make the interesting clinical comment that "In at least three recent instances such a finding in patients originally suspected of an acute surgical abdomen assisted in steering the therapy along non-surgical lines".

In the case of measles the prodromal stage, when the virus is multiplying in the body, is associated with lymphoid tissue hyperplasia and a specific giant-cell reaction. These cells, the Warthin-Finkeldey cells, were described independently by Warthin and by Finkeldey in 1931. Warthin described them in the pharyngeal mucosa and the tonsils. Hathaway (1935) reported their occurrence in spleen and lymph nodes. Gordon and Knighton (1941) obtained blood from patients suffering from measles and injected it intravenously into a number of experimental animals, including 4 monkeys. Inguinal lymph nodes were removed immediately before the injection and at varying periods afterwards. As compared with the pre-inoculation node all the others "revealed some

degree of hyperplastic lymphadenitis", and in 3 monkeys out of the 4 Warthin-Finkeldey giant cells were found.

Interrelationship of small, medium and large Lymphocytes

From the evidence so far presented two main points emerge. First, lymphocytes are produced normally in large numbers, and second, although various suggestions as to their function have been advanced, none are particularly convincing. The lymphocyte seems to be almost ubiquitous and to be involved in the response to such a variety of pathological conditions that it is difficult to conceive of any function common to all these lymphocytic reactions. We are therefore compelled to consider another possibility, i.e. that the lymphocyte may be important not for what it does, but for what it is Or in other words, the lymphocyte itself may not possess any important functions, but it may be capable of becoming transformed into other cells which do. Much of the evidence bearing on this, the most controversial aspect of the problem of lymphoid tissue, is primarily morphological. Although the literature is very extensive, it has been fully reviewed by Bloom (1938*a*, *b*, and *c*), and later by Rebuck and Crowley (1955), so that we need not go over the whole ground again. Our chief concern here is with the method of approach and with certain trends which have perhaps not been sufficiently emphasized.

We must first *consider the relation between the small, medium, and* large lymphocyte, for it has been suggested that there is evidence of orderly maturation within the lymphocyte series, without any transformation into other cells Examination of sections of lymphoid tissue, of fixed smears, or of supravital preparations reveals the existence of many transitional forms between the various kinds of lymphocyte, so that there is no doubt that one type has changed into the other. What is not so clear, however, is the direction of the change. It seems to be generally agreed that the larger lymphocytes can change into the smaller; but is the change reversible and can the small lymphocyte grow into a large one? Maximow (1927) and his followers consider the three forms interchangeable and capable of developing one into the other Other workers, such as Wiseman (1931*b*, 1931–1932), who suggests that the small lymphocyte may be the mature end stage of the lymphocyte cycle, maintain that the process is one-way and that small lymphocytes cannot become large ones

Virtually all observers are agreed that large and medium lymphocytes can undergo mitosis, and in the germ centres of lymph glands it is mainly in the medium lymphocytes that mitoses are to be observed. Small lymphocytes on the other hand appear to undergo mitosis very infrequently, though they are said to contain the normal diploid number of chromosomes (Petrakis, 1953). In the lymphoid tissues it was in the thymus alone that

Kindred (1938) could observe small lymphocytes in mitosis and even then not very often. Trowell (1952) illustrates what he described as small lymphocytes in mitosis in cultures of rat lymph node, but gives no further information We ourselves, in the study of lymphocytes in both lymphoid tissue and marrow suspensions taken from several hundred guinea-pigs, have rarely seen anything which could be definitely interpreted as a small lymphocyte in mitosis. Leitner (1949) stresses this point for human marrow. Sundberg (1955) does find occasional mitoses in what may be small lymphocytes in human marrow, but emphasizes too that mitoses are conspicuous by their absence in the large perivascular aggregates of small lymphocytes On the whole, then, it may be said that mitoses in small lymphocytes are few and far between.

What precisely does maturation mean ? In the case of the erythrocytes and granulocytes it involves the specialization of the not inconsiderable cytoplasm with the development of haemoglobin or specific granules. Together with this there are degenerative changes in the nucleus, proceeding in the mammalian erythrocyte to its complete disappearance. In the case of the small lymphocyte there is no clear-cut evidence of cytoplasmic specialization If the small lymphocyte matures from the medium and large lymphocyte, then the maturation involves, so far as we now know, not the specialization of the cytoplasm but its diminution and atrophy. It is therefore of a unique type, fundamentally different from that of the other blood cells. In the absence of any of the usual criteria, Wiseman (1931*b*) has stressed the significance of decreasing basophilia as an index of senescence But even he finds it of doubtful value, for "although basophilia is manifestly a criterion of youth, the absence of it in a given cell does not necessarily mean that the cell is mature"

In recent years the significance of cytoplasmic basophilia has been studied intensively, both by histochemical and histophysical methods, the latter in the [illegible]

[illegible] Caspersson, 1950) Basophilia is believed to accompany new protein formation and this as a rule is an indication of cell growth, though in the case of the plasma cell the protein gamma globulin is formed as a cellular secretion If we accept the basic soundness of Caspersson's view, we are still left with the possibility that basophilic cells may arise from non-basophilic precursors, as for example when the primitive reticular cell in lymphoid tissue, which is not basophilic, transforms into the lymphoblast and large lymphocyte, which are.

Thorell (1945), comparing the ultraviolet absorption of small and large lymphocytes, inferred from the nucleic acid content that small lymphocytes possess a very low growth rate and are therefore to be regarded as mature

cells. In this he differs from Wiseman (1931*b*) who, using both fixed smears and supravital staining, concludes that "the small lymphocyte may be of any age in specimens examined by either technique", and that "*no one criterion enables one to pronounce upon this point*", *i.e.* whether a lymphocyte is mature or not. Ackerman and Bellios (1955) are also of the opinion that cell size is not a decisive criterion of maturity.

The nucleus of the small lymphocyte has been held to show signs of degeneration in the form of chromatin condensation, and it is of course true that the nucleus as a rule is characteristically pachychromatic. In addition, it is also suggested that the lack of a nucleolus is evidence of maturity. Apart from the fact that the significance of the nucleolus is far from certain (see Cameron, 1952), it is unfortunate that most workers have endeavoured to demonstrate nucleoli in air-dried smears, whereas they can best be shown following wet fixation, when as pointed out by Maximow (1932) and others most lymphocytes can be shown to possess nucleoli, except immediately before or after mitosis. In supravital preparations the nucleolus stains readily with brilliant cresyl blue, and in smears it stains clearly with methylene blue at a *p*H 4 9 (Stockinger and Kellner, 1952) According to Pulvertaft and Jayne (1953) as well as Ackerman and Bellios (1955) the nucleoli can be seen by phase contrast microscopy

Cunningham, Sabin and Doan (1925) held that "the process of maturation of the lymphoblast into the adult lymphocyte is largely evidenced by a gradual concentration of the mitochondria", with a diminution in their number. It is questionable, however, whether mitochondria really possess this significance. Lewis and Lewis (1914–1915), after a detailed study of mitochondria in tissue cultures, concluded that "the mitochondria are extremely variable bodies, which are continually moving and changing shape in the cytoplasm"; while the experiments of McCurdy (1939) showed that the shape and distribution of the mitochondria are functions of the nutritive condition of the cell.

Cunningham, Sabin and Doan (1925) believed that the appearance of neutral red bodies in the lymphocyte was evidence of maturation, though later workers rejected this view (Hall, 1938, Schwind, 1950). According to Wiseman (1931–1932): "Neutral red vacuoles when present [in the lymphocyte] are distributed without pattern, are usually few in number, and without significance as to the age of the cell They therefore represent degenerative or functional vacuolization and bear no relationship to age or identification of cell type."

The neutral red granules are not to be confused with the occasional [illegible] nt in 34 per [illegible] hought that [illegible] irradiation, [illegible]

though Watts and Matheson (1950) were unable to confirm this finding. Ackerman and Bellios (1955) describe the occasional presence in the lymphocyte of a small highly refractile non-staining lipoid droplet near the cytocentrum on phase contrast examination.

Lymphocytes contain a cytocentrum (Weidenreich, 1911; Wallgren, 1911, Ackerman and Bellios, 1955)

There is some interesting embryological evidence. Gilmour (1941) found that in human embryos lymphopoiesis was evident in the connective tissues at the 26 mm. stage, and in lymph glands at 48 mm., at which time the lymphatic vessels too already contained some lymphocytes. Lymphocytes began to appear in the thymus of the 35 mm. embryo and in the bone marrow in embryos of 48-65 mm. In chick embryos Sabin (1922) reported that "the lymphocyte appears in the circulating blood on the fifth day, in its smallest form, an occasional one may occur on the fourth day. . . . In the stained blood smears, the lymphocytes are easily distinguishable by their characteristic nuclei and the fact that they are all at first small lymphocytes" It is difficult to reconcile this finding with the view that the small lymphocyte is a mature form of the large, for it hardly seems possible that the mature form should be the first to appear

The view that the small lymphocyte is a mature cell is thus based on very unsatisfactory evidence. But ideas die hard and the concept is still widely quoted in haematological literature.

Some of the most convincing evidence that small lymphocytes can grow into medium and large lymphocytes is based on tissue culture experiments, more particularly those in which a practically pure suspension of lymphocytes was used to begin with, such as thoracic duct lymph (Bloom, 1937), or the buffy coat in chronic lymphatic leukaemia (Pierce, 1932). This evidence would be decisive, were it not for the fact that in the hands of many workers the small lymphocyte in tissue culture has been found to remain alive and active for several days, but not to grow in size or to differentiate Thus Medawar (1940) repeated the experiments of Bloom (1937) but could not confirm his results Though Medawar departed from Bloom's technique in some important particulars, he nevertheless concluded that Bloom's results were due to contamination of the lymph with other cells.

Ebert, Sanders and Florey (1940) observed individual lymphocytes continuously for as long as 24 hours in transparent chambers in the rabbit's ear and saw no evidence of their transformation into any other cell type. However, their work did bring out one fundamental point Lymphocytes from a fragment of a popliteal node (autogenous) were observed alive and motile, in the neighbourhood of blood vessels, in one case for 7 days, while in yet another many lymphocytes were still present after 26 days.

Their work appears definitely to disprove the view that the lymphocyte lives for only a few days, and offers positive evidence, based on direct observation, on the length of life of lymphocytes *in vivo*.

One cannot help feeling, when reading the various tissue culture reports (for bibliography see Bloom, 1938*c*, Rebuck and Crowley, 1955) that in tissue culture as at present performed there are still too many unknown variables Oxygen content may possibly be a factor of some importance. Thus Trowell (1955), working with cultures of rat lymph nodes in pure oxygen, found that in a simple synthetic medium he could obtain not only survival of small lymphocytes, but also their differentiation into macrophages. Foreign proteins may also act as a stimulus. Pulvertaft and Jayne (1953) cultivated lymphocytes from a malignant exudate on the surface of an agar plate and found the cells lived for over a week, moved actively, and were capable of mitosis throughout the observation period.

That lymphocytes can hypertrophy and become transformed into macrophages during aseptic inflammation was demonstrated by Maximow in 1902 by the insertion of celloidin chambers into the tissues Since then many workers have obtained similar results and we shall here refer only to a few recent papers.

Kolouch (1939) produced an aseptic inflammation in rabbits by the subdermal injection of egg albumin and then examined stained dry spreads of connective tissue removed from the site of injection at varying periods afterwards He recorded that 8 hours after the albumen injection the inflamed area showed numerous typical small lymphocytes, while by the fourteenth hour many of these were becoming transformed into macrophages Berman (1942) devised a technique for obtaining dry films of tissue cultures of rabbit lymph nodes by the simple expedient of blotting the culture to remove excess of cells and fibrin. By 31 hours there was clear evidence of lymphocyte transformation into macrophages The nucleus at first became leptochromatic and subsequently developed a nucleolus Rebuck (for details see Rebuck and Crowley, 1955) has made use of a most ingenious method for obtaining regular samples of tissue lymphocytes in man from a region of aseptic inflammation. At 12 hours after the commencement of the inflammation there was clear evidence of lymphocytes beginning to undergo hypertrophy and become macrophages. Again the change from the pachychromatic to the leptochromatic type of nucleus was one of the characteristic features of the process.

Lymphocytes in Circulating Blood

If the small lymphocytes are capable of growth and if they grow appreciably while in the blood, it would seem worth while to compare the sizes of the thoracic duct and blood lymphocytes. Such a comparison

TABLE 51

Number of lymphocytes of various sizes found in counting 100 white blood cells in supravital and fixed preparations of blood from five normal human adults

Blood specimen	*Technique*	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13	13–14	14–15	15–16	16–17	17–18	18–19	19–20	20 +
		Gross cell diameters in micra															
R 1497 Female—age 58	Supravital	—	6	13	3	4	1	—	—	—	—	—	—	—	—	—	—
	Fixed	—	—	—	1	8	1	4	3	2	6	2	—	—	—	—	—
R 1498 Male—age 28	Supravital	—	—	6	8	11	6	1	1	—	—	—	—	—	—	—	—
	Fixed	—	—	—	4	5	4	10	7	0	2	1	—	—	—	—	—
R 1499 Male—age 31	Supravital	1	2	2	3	6	0	2	—	—	—	—	—	—	—	—	—
	Fixed	—	—	—	—	1	2	4	6	1	2	1	—	—	—	—	—
R 1500 Female—age 27	Supravital	—	3	9	1	8	1	3	1	0	1	—	—	—	—	—	—
	Fixed	—	—	—	1	3	4	10	6	0	2	1	—	—	—	—	—
R 1501 Male—age 33	Supravital	2	4	8	6	6	0	4	0	0	1	—	—	—	—	—	—
	Fixed	—	—	—	1	5	2	6	6	2	6	2	0	0	0	2	—
Totals	Supravital	3	15	38	21	35	8	10	2	0	2	—	—	—	—	—	—
	Fixed	—	—	—	7	22	13	34	26	5	18	7	0	0	0	2	—
Per cent	Supravital			83			13						4				
	Fixed			20			35						45				

From Wiseman (1931*b*)

(Table 51) does not, however, throw much light on the problem. For while it is true that the blood lymphocytes, when examined in fixed smears, are somewhat larger than those in the thoracic duct lymph from which they came, this size *difference cannot be accepted as conclusive proof of* growth. Lymph, owing to its lack of body, does not give such thin films as blood and the cells are not so well stretched on the slide, except at the edge of the film In fixed smears of blood, on the other hand, stretching

TABLE 52

Percentages of lymphocytes of different sizes measured in supravital preparations of normal human blood and of the blood of normal newborn and adult rabbits

Animal	Lymphocytes *Small* %	*Intermediate* %	*Large* %
Man	82	17	1
Adult rabbit	90 5	8 1	1 4
Newborn rabbit	85 7	13 6	0 7

Data from Wiseman (1931*b*)

exaggerates the size of the cells (see Wiseman, 1931*b*) If one reckons cells below 10 μ as small lymphocytes, from 10 to 12 μ as intermediate, and from 12 μ upwards as large lymphocytes, then, according to Wiseman, in fixed smears only 20 per cent of the blood lymphocytes are small, as against 83 per cent in supravital preparations (Table 51) It is interesting to note that approximately the same percentage of small lymphocytes is found in the blood of human beings and of newborn and adult rabbits (Table 52). With this type of evidence before us that the majority of the lymphocytes in the blood are small (80 or 90 per cent as seen in supravital preparations), it is clear that when we consider the fate of the blood lymphocytes it is primarily the small lymphocyte with which we are concerned.

RELATION OF LYMPHOCYTES TO OTHER CELLS

The question of the genetic relationship between lymphocytes and *other cells may be considered under three main headings* (1) criteria for distinguishing lymphocytes from other cells, (2) the origin of lymphocytes from fixed tissue cells; and (3) the transformation of lymphocytes into plasma cells, monocytes, macrophages, fibroblasts, or stem cells of the erythrocyte or granulocyte series (myeloblast, primitive cell, and pro-erythroblast) The problem would be simple if we possessed any clear-cut criteria for distinguishing lymphocytes from other cells. A review of the literature, however, shows that many criteria have been put forward, only to be discarded as further analysis has shown them to be unsatisfactory.

CRITERIA FOR DISTINGUISHING LYMPHOCYTES FROM OTHER CELLS

Motility and Absence of Granules Ehrlich and Lazarus (1905) believed that lack of motility and absence of granules were fundamental criteria for distinguishing lymphocytes. We now know that lymphocytes can move, and that they may occasionally possess azurophilic granules (Michaelis and Wolff, 1902), though it is questionable whether these granules are comparable in significance with those found in granulocytes. In any case, if the lymphocyte is a primitive cell with multiple developmental potentialities, the absence of granules—which might be regarded as a sign of commencing specialization—would not be altogether unexpected.

Mitochondria. Schridde (1907), using a modification of Altmann's technique, described granules which he thought were peculiar to cells of the lymphoid series It has subsequently been shown, however, that the Altmann-Schridde granules are mitochondria, which are also present in myeloid cells (Cunningham, Sabin and Doan, 1925). For further details concerning mitochondria and their significance in haematology, the reviews by Hall (1938) and Schwind (1950) may be consulted

Neutral Red Rosette Renaut and Dubreuil (1906) described the presence in living cells of granules which could be stained by neutral red. Simpson (1922) considered that the number and arrangement of these neutral red granules (the "rosette" of Sabin, Doan and Cunningham, 1925) were distinctive for the monocyte, and could be used to separate it definitely from other cells, including the lymphocyte Carrel and Ebeling (1926) concluded that the rosette could only be found in the inactive monocyte "The monocytes observed during the first hours of cultivation possessed the appearance that characterizes these cells and manifested the well known reaction to neutral red The granules stained by neutral red are small and move swiftly within the cells They are scattered in the cytoplasm, without forming the rosette represented in the drawings of Sabin In the cultures that have been kept at a lower temperature, and where the cells are less active, the rosette becomes apparent." Cappell (1929) finds that some monocytes contain definite rosettes, but "the majority of these cells in my experience do not exhibit such a regular cytoplasmic pattern, the granules do not always form a rosette but may be scattered through the cytoplasm, and their colour does not always present any noteworthy difference from that of the granules of the lymphocytes and other cells" Wiseman (1931–1932) pictures a lymphocyte with a typical neutral red rosette but comments "Although the arrangement of neutral red bodies in the cell of Fig 1 might be confused with the rosette of a monocyte, yet there are other criteria that definitely place this cell in the lymphocyte group" It is true that the

rosette is found most frequently in the monocyte, but it can also occur in lymphocyte, plasma cell, macrophage, and epitheloid cell; and, from the point of view of cell classification therefore, has no decisive significance (see also Schwind, 1950)

Oxidase Reaction. It was formerly thought that whereas all cells of the lymphoid series were oxidase-negative, myeloid cells were oxidase-positive. This, if true, would be an invaluable criterion for distinguishing lymphoid from myeloid cells. It has been shown (Menten, 1919; Richter, 1925, Sabin, 1928; Sabin *et al.*, 1924), however, that although granulocytes are oxidase-positive from the myelocyte stage onwards, the myeloblast itself is oxidase-negative; and it is precisely at the myeloblast stage that the separation of lymphoid from myeloid cells becomes so difficult and controversial Again the argument is two-edged. If the lymphocyte is a primitive cell and oxidase granules develop in cells arising from lymphocytes as they undergo specialization, the absence of a positive reaction in the lymphocyte itself is only to be expected. In this connection it is of interest to note that lymphocytes appear in the chick embryo on the fourth day (Sabin, 1922), but the first oxidase-positive cells are not found until the twelfth day (Silberberg and Orzechowski, 1928). The intracellular respiratory pigment, cytochrome, which is usually associated with oxidase, is also not present in lymphocytes (Faerber, 1939)

Origin of Lymphocytes from fixed Tissue Cells

Lymphocytes can multiply by mitosis of pre-existing lymphocytes. There is also some evidence that they can arise from the fixed reticular cells of lymphoid tissue (Downey and Weidenreich, 1912), but whether all the reticular cells are potentially equal in this respect is not known Maximow (1927) divides reticular cells into two groups, one of which has already undergone partial specialization and can take up vital dyes, the other is more primitive and does not ingest dyes. It is this latter group alone, according to Maximow, which is capable of becoming transformed into large and medium lymphocytes. Jaffe and Richter (1928) found, in autoplastic transplants of lymph nodes in the rat, that most of the lymphocytes in the transplants degenerated, whereas the reticulum cells underwent hyperplasia, and gave rise to fresh lymphocytes. " . . . the hyperplastic reticulum appears to be the important source of lymphocyte production. These may be derived from the reticulum directly as small lymphocytes, or may be formed through the intermediary stage of large lymphoid cells."

Transformation of Lymphocytes

Plasma Cells

That the lymphocyte can become transformed into a plasma cell has long been accepted by many observers. "The one perfectly clear fact is that a plasma cell is a modified lymphocyte. That this modification represents progressive differentiation or signifies specific function seems doubtful" (Jordan and Morton, 1937) On the other hand Miller (1931) is equally emphatic: "This . makes it clear that plasma cells are not derived from lymphocytes."

In recent years the association of the plasma cell with antibody formation has stimulated further interest in its histogenesis. An excellent discussion of the problem will be found in the review by Sundberg (1955), who states that. "Plasmablasts, haemopoietic reticular cells, and reticular lymphocytes are morphologically distinguishable from one another, but often their similarities are greater than their differences." Braunsteiner, Fellinger and Pakesch (1953) were able to show, with the aid of the electron microscope, that the plasma cell has a very characteristic lamellar structure in its cytoplasm. Here again, however, the earlier stages presented difficulty, and Braunsteiner and Pakesch (1955) would not venture to distinguish definitely between the plasmablast and the undifferentiated lymphoid reticular cell. Moeschlin and Demiral (1952) refer to the earlier investigations of Moeschlin and his co-workers, and report the occurrence in the cytoplasm of plasma cells of granules which could be seen on phase-contrast examination Jordan (1954) re-affirms his earlier views about the transformation of lymphocytes into plasma cells and maintains that the precursors of both lymphocytes and plasma cells are identical

Monocytes

In normal blood the typical small lymphocyte and the monocyte are readily distinguishable The N C (nucleus to cytoplasm) ratio is perhaps the most obvious single criterion In some animals the nuclear structure may also be a valuable guide Difficulty arises with the medium and large lymphocytes, where the N C ratio loses its diagnostic value, while at the same time the nuclear structure of the lymphocyte becomes finer, approximating more closely to that of the monocyte In consequence there is always a smaller or larger group of cells which it is impossible, on morphological grounds, to classify as lymphocytes or monocytes, and "this indeterminate group must appear as a transitional group linking the lymphocytes with the monocytes morphologically" (Bloom, 1938*a*, cf Cappell, 1930)

Macrophages and Fibroblasts

The evidence of tissue culture experiments for the possible transformation of lymphocytes into monocytes is much more extensive and

rosette is found most frequently in the monocyte, but it can also occur in lymphocyte, plasma cell, macrophage, and epitheloid cell; and, from the point of view of cell classification therefore, has no decisive significance (see also Schwind, 1950).

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nucleoli and thin nuclear membranes were more frequent in the cells from the marrow there was nothing to distinguish marrow cells from lymphocytes" The same results were obtained by the moist smear technique, but quite different ones when the marrow and lymph node preparations were made by the dry smear method. In dry smear preparations cytoplasmic differences are valueless, except where the myeloblast has already begun to develop many azurophilic granules, but, according to Downey, "the nuclei [of the myeloblast] are very different from any seen in the human or rabbit lymphocytes, the chief characteristics being the diffuse and rather uniform distribution of the chromatin which occurs in the form of fine dust-like particles and the extremely thin nuclear membrane. There is some tendency for the chromatin to concentrate about the nucleoli, but otherwise its distribution is rather uniform, except . . ." It is this "except" which one comes up against so [illegible]

immature." It is difficult to know precisely what is meant by an immature myeloblast. However, it must be admitted that in the usual air-dried films the larger myeloblasts of bone marrow can in most cases, though not in all, be distinguished from the blast-like cells of lymphoid tissue

The Micromyeloblast

But the position is very different in the case of the micromyeloblasts Naegeli, who first described the myeloblast as a distinct cell type, repeatedly states that the micromyeloblast is very like the small lymphocyte, and leaves it at that. If micromyeloblast and small lymphocyte, despite their similarity, really are distinct cell types, then the first question which presents itself is What is the origin of the micromyeloblast? It could be derived from the larger myeloblast by mitosis, and this is the origin attributed to them when the problem is faced (e.g. Sabin *et al.*, 1924). But Naegeli (1931) does not consider the origin of the micromyeloblast, and merely comments that it may be either an embryonic or a pathological cell type

In fact, the derivation of the micromyeloblast from the myeloblast is beset with several difficulties. Though admittedly the cytoplasm would diminish on mitosis, it should not be quite as scanty in the micromyeloblast as it actually is (cf. Naegeli, 1931, Abb 67, cells 2 and 3), while on the other hand one could expect it to show the same intensity of basophilia as the myeloblast, which it frequently does not It was no doubt considerations such as these which made Naegeli incline to the view that the micromyeloblasts might be abnormal—either embryonic or pathological cells Furthermore, myeloblasts are comparatively few in healthy marrow

convincing than it is for their change into myeloid cells. This evidence is the more significant, since it is now generally held that the monocyte can become a macrophage and possibly a fibroblast. Maximow (1928), observing the transformation of blood lymphocytes into macrophages, believed that they usually passed through a typical monocytoid stage. Cappell (1930) investigated the cells appearing in the peritoneal exudate following mild irritation of the peritoneum. He found that " By the end of six to nine hours, . . the lymphocytic cells . . exhibit commencing neutral red storage ", and after twelve hours show clear evidence of nuclear enlargement and indentation. " No clear distinction can be made by supravital staining between lymphocytes, monocytes, and macrophages; while typical examples of each can readily be identified, the three types are connected by all intermediate stages, and it is clear that a progressive development is going on in the smaller vessels, by which they are rapidly acquiring the functional characters of young macrophages."

Considerable attention has been devoted to the histology of the lymphoid tissues in infections with *B monocytogenes*. Witts and Webb (1927) could not find any evidence of monocytes arising from lymphocytes. Others have observed this transformation extensively. Conway (1938) reviews the literature, and sums up her own observations by saying: " The free stem cell from which the monocyte develops in the diffuse lymphatic tissue and lymphatic nodules of the mesenteric lymph node and in the spleen is morphologically identical with the lymphocytes in these tissues "

Myeloblasts

Since Naegeli (1900) first described the myeloblast, the relation between it and the lymphocyte has been the subject of controversy. " Die Grosse der Myeloblasten schwankt von Lymphocytengrosse (und dann Lymphocyten sehr ahnlich) bis zur Myelocytengrosse . . " Confusion arises between the smallest myeloblasts (Naegeli's " Mikromyeloblasten ") and the small lymphocyte, and between larger myeloblasts and the medium and larger lymphocytes, especially those found in the germinal centres of lymph nodes and often termed " lymphoblasts ". The smaller myeloblasts are extraordinarily difficult to distinguish from the small lymphocyte. As to the larger myeloblasts, Downey (1938), writing of his studies of bone marrow and lymph nodes prepared by the section method, " If the conclusions were based on the sections alone they would have to be of the extreme unitarian variety, for it was absolutely impossible to demonstrate any constant differences between the marrow cells and the larger lymphocytes of the nodes Particular attention was given to the structure of the nuclei because the nuclei furnish the best means of separating the cells in the blood smears But outside of the fact that the multiple

TABLE 53

Comparison of right and left humeral marrow in guinea-pigs (all counts are in absolute numbers per c.mm.)

	Mean	*Standard deviation*	*Standard error of the mean*
Right			
Erythroid . . .	272,220	96,070	23,240
Myeloid	389,690	130,000	31,460
Lymphocytes . .	301,130	170,470	25,460
Monocytes . . .	19,940	12,570	3,050
Damaged . . .	316,790	117,730	25,550
Total abs count .	1,402,220	240,500	58,330
M : E ratio . .	1·53	·46	·159
Left			
Erythroid . . .	273,160	73,250	19,160
Myeloid	356,500	132,000	34,080
Lymphocytes .	326,000	101,600	26,230
Monocytes . . .	30,950	20,000	5,160
Damaged. .	291,430	116,000	30,130
Total abs. count .	1,383,810	266,000	66,500
M · E ratio . . .	1·45	·48	·12

After Harris, Herdan, Ancill and Yoffey (1954)

(Yoffey, 1955). Here the marrow contains about 300,000 lymphocytes per c.mm. marrow (Harris *et al.*, 1954), and photomicrographs of these cells in colour are given in the paper by Yoffey *et al*. (1954) Most characteristically, the small lymphocytes in bone marrow have only a very small amount of cytoplasm, situated at one pole of the cell, so that we sometimes refer to them as "polar". These cells are remarkably like the cell figured by Naegeli (1931) in his Abb. 67, cell 3 But their pachychromatic nuclei cannot be explained by faulty preparation of the smear, and they occur in such large numbers that even from a purely quantitative point of view they cannot possibly be derived from the large myeloblasts. In the marrow of these guinea-pigs the myeloblasts form about 1–2 per cent of the total nucleated cells, and the small lymphocytes about 20 per cent. The lymphocyte . myeloblast ratio is therefore about 15 · 1 and if the former were derived from the latter, myeloblasts should be seen frequently in mitosis, which as already pointed out is not the case , mitoses among myeloblasts are most infrequent.

Furthermore the micromyeloblasts—if we call these cells by that name —are not merely smaller versions of the myeloblast The cytoplasm usually has only a moderate degree of basophilia, of the same order of intensity as is found in the small lymphocyte elsewhere. If these " micromyeloblasts " are formed by the division of the larger myeloblasts, we would expect them to have reduced cytoplasm immediately after the mitosis, yet none the less a good deal more than is possessed by the polar

—so much so that Rohr (1949) considers that they can play little part in normal blood formation. Not only are the myeloblasts few in number, but mitoses in them are not at all frequent (cf. Leitner, 1949). A similar problem arises if one postulates an origin for the micromyeloblast from the fixed (reticulum) cells. These again are few in number, and also (Leitner, 1949) rarely undergo mitosis

The paucity of the blast cells is one which repeatedly impresses itself upon haematologists. Thus in addition to the observations of Rohr (1949) Bloom (1948) comments: " . . . haemocytoblasts are exceedingly rare in normal rabbit marrow." Sundberg, Schaar and May (1952) in discussing monkey marrow observe: " One is continually amazed in studying normal marrow that pro- and basophilic normoblasts can function as precursors for cells which must form such large numbers of erythrocytes to meet the normal demand." In short, there are singularly few blast cells to give rise to the granulocytes and erythroid cells in the numbers required. If in addition they must give origin to large numbers of micromyeloblasts, the problem becomes very much more difficult.

But the problem becomes simplified if the micromyeloblast is in fact a small lymphocyte One could thus account for the fact that many of the cells which have been termed micromyeloblasts possess so little [illegible]

(p 208, Abb 67, cells 1–3) Cell No. 1 is quite large for a [illegible] —and incidentally none of the three cells possesses a nucleolus—while cells 2 and 3 are the size of a small lymphocyte, but cell 3 has a pachychromatic and cell 2 a leptochromatic nucleus. Naegeli evidently had qualms about the pachychromatic nucleus, for he comments, " Das L.-ähnliche Aussehen des Kernes bei Mikromyeloblasten ist aber oft nur durch schlechte Ausbreitung bedingt." Naegeli was not alone in experiencing this difficulty, and we would add in passing that we do not believe this explanation to be generally applicable. Schulten (1937) also illustrates (Tafel XIIIC) " Myeloblasten von Lymphozyten nicht sicher zu unterscheiden ", even though the nuclei are also markedly pachychromatic. By other haematologists these cells are sometimes referred to as haematogones, e g Vogel and Bassen (1939) who term them " the mystery cells of the bone marrow ", and Glaser, Limarzi and Poncher (1950) who state however that " statistically they are insignificant ". Whatever the position as far as human marrow is concerned, it can be asserted quite unequivocally that in guinea-pig marrow the number of these cells is highly significant. Gardikas and Israels (1948) have made use of the Feulgen reaction in an endeavour to distinguish between lymphocyte and micromyeloblast.

The problem can be seen in very much better perspective with the aid of quantitative data, such as are now available in the guinea-pig

Formationen liegen aber extraparenchymatisch und sind normal nur minimal entwickelt in der Nahe der Gefasse." It is difficult to know what is meant by the statement that lymphoid tissue—and presumably scattered lymphocytes—in the bone marrow are extraparenchymatous But no doubt because it had the weight of Naegeli's authority behind it the statement has often been repeated in the haematological literature. It cannot be too strongly emphasized, therefore, that in normal marrow there are considerable numbers of small lymphocytes, that they are dispersed throughout the marrow freely intermingled with the other marrow cells, and they are as much a part of the marrow parenchyma as any of the other cells of which the marrow is composed.

Primitive Cells

In 1925 Cunningham, Sabin and Doan, relying mainly on the supravital staining technique, arrived at a new theory of blood formation in which the ancestor of all the white cells was the "primitive" cell "These primitive cells are usually small, with a centrally placed nucleus containing a definite nucleolus; their cytoplasm is like heavy ground glass and contains a few large, evenly distributed mitochondria which always stain with janus green. These mitochondria form no pattern whatever, and, while they are usually blunt rods, they may occasionally be large, heavy droplets These primitive cells are not very numerous; they are pretty evenly scattered throughout the marrow and never contain anything that stains with neutral red" It was maintained at first that the primitive cell and the small lymphocyte were readily distinguishable, but subsequently this point of view underwent progressive modification. " . . this cell, though it looks much like the small lymphocyte, lacks certain signs of differentiation. The differences though meagre are worthy of consideration" (Sabin *et al*, 1936) Further " It is quite clear that the occurrence of any granulations in these primitive types may be interpreted as evidence of the origin of granulocytes from lymphocytes and we do not wish to stress a difference without a distinction, nor one which is merely to be resolved by terminology" In a still later paper Sabin and Miller (1938) conclude that, though the morphological criteria for distinguishing primitive cell and small lymphocyte are not satisfactory, it can be shown on other grounds that the small lymphocyte is a mature functioning cell These grounds are. first, the experiments of Wiseman (1931*b*) on the lymphocytic response to foreign protein; second, the fact that the lymphocyte is involved in the response to tuberculous infection.

Cunningham, Sabin and Doan (1925). A critical review will be found in

small lymphocytes. In addition, mitosis should not result in a lessening of the cytoplasmic basophilia.

TRANSITIONAL LYMPHOCYTES

At this point it is pertinent to observe that we do in fact observe transition forms between the small lymphocytes and the blast cells. There are always transition forms present even in normal marrow, but they increase appreciably in number when the marrow is stimulated to increased red cell or granulocyte formation. In the transitional lymphocytes, as we now term them, the first change is in the nucleus, which shows a progressive leptochromasia gradually replacing the pachychromasia. Naegeli's (1931) Abb. 67, cell 2, appears to be a typical cell of the kind which in guinea-pig marrow we describe as a "transitional" lymphocyte, and as compared with his cell No 3 it shows very well the leptochromatic change in the nucleus After the nuclear change the cell appears to enlarge rapidly, still retaining a high N.C ratio. It is only after the nucleus has enlarged considerably, as a rule, that the cytoplasm begins to increase; until the basophilia is well advanced there is usually no sign of a nucleolus in air-dried films, and often not even then. There may however be a so-called chromocentre, which is often visible in the air-dried smear (see Yoffey *et al.*, 1954, Plate I) During the enlargement of the cell the nucleus frequently shows indentations which are reminiscent of the Rieder cell of human marrow. But we agree with Sundberg (1942) that these indentations occur in healthy guinea-pig marrow and are not to be regarded as pathological

It is clear, of course, that the presence of numerous transition forms between blast cells and small lymphocytes could also be interpreted to indicate a heteroplastic formation of the latter from the former. But we have not accepted this interpretation, partly for the reason already given, that the marrow seems to possess far too few blast cells to account for its need of red cells and granulocytes, without adding lymphocytes also, and partly because of the quantitative relationships which exist between marrow and blood lymphocytes, discussed on p. 336.

One final point needs to be made in discussing the relation between the small lymphocyte and the micromyeloblast. Naegeli's views on blood formation were such that the lymphocytes had no obvious rôle to play in bone marrow. This may have been partly responsible for his describing many of the small lymphocytes in bone marrow as micromyeloblasts. However, the fact nevertheless remained that there are lymphocytes in the marrow. "Freilich ist auch eine Existenz lymphatischer Bildungen in Knochenmark sichergestellt." But Naegeli got over this difficulty by putting the lymphocytes outside the marrow parenchyma "Diese

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the paper by Schwind (1950), who after careful study concluded that he was unable by the supravital technique to distinguish between lymphocytes [illegible] l cells as seen [illegible] of haemato-

Pro-erythroblasts

The red cell precursors (pro-erythroblasts or erythrogones) present the same kind of difficulty as the myeloblasts, but the problem is limited to their very early stages. Once maturation begins, the intense basophilia and associated nuclear changes leave no doubt as to whether one is dealing with pro-erythroblast or lymphocyte.

Summarizing the preceding section, we may say that tissue culture experiments suggest that lymphocytes—including the small lymphocyte—are capable of growth and development. It is noteworthy also that in the blood and blood-forming organs it is difficult to draw a rigid line of separation between lymphocytes and other cells—monocyte, myeloblast, primitive cell, and erythrogone. There are always to be found a certain number of cells which can be interpreted as transition forms. In the case of some myeloblasts and of all primitive cells, there seems to be complete morphological identity with members of the lymphocyte series. This is forced upon our attention more particularly in studies of bone marrow. Throughout the literature on the cellular composition of marrow, one is impressed by the frequency with which investigators describe the presence of "lymphoid" cells, cells very like lymphocytes, but showing some minute differences, whose precise significance is difficult to evaluate.

Lymphocytes in Pathological Conditions

Lymphocytes appear in increased numbers—either in the tissues, in the blood, or in both—in response to a wide variety of pathological conditions. The accumulation of small lymphocytes, the classical small round cell infiltration, is noted so regularly in the tissues around tumours that one observes it without special interest. In many of the chronic inflammatory conditions—tuberculosis and syphilis are perhaps the most outstanding examples—small lymphocytes may appear on the scene in large numbers In virus diseases they often have a characteristic perivascular distribution. Lest one be tempted, however, to think that lymphocytes are largely concerned with the reaction to bacteria and viruses, it should be noted that they are also found in increased numbers in aseptic wounds (cf. Cruickshank, 1942) In the absence of more definite knowledge of lymphocytic function, a rational account of the lymphocyte in

pathological conditions is impossible. If one accepts the view that lymphocytes can develop into monocytes, macrophages, and fibroblasts, an easily intelligible rôle can be assigned to them in the phenomena of inflammation and repair. Otherwise, we are unable to account for their presence, although numerous hypotheses, some of which we have previously mentioned, have been put forward.

Changes in number and quality of the blood lymphocytes have been described in a number of bacterial and virus diseases. These changes tend to be more marked in children and give rise to the term "lymphatic reaction". But since in these cases we do not know the number of lymphocytes entering the blood or the number leaving it, the significance of changes in the blood lymphocytes cannot be fully appreciated, even from a purely quantitative point of view and quite apart from the question of function. It may be that lymphocytosis is partly a non-specific response *to the presence of bacterial or virus protein, especially in view of Wiseman's* (1931*a*) experiments on the parenteral injection of foreign protein. In some cases, however, it seems likely that there is a specific stimulus to the lymphoid tissues In whooping cough, for example, the lymphocytosis may attain almost leukaemic dimensions.

From a clinical point of view, there is one very noteworthy feature about changes in the number of blood lymphocytes. Generally speaking, lymphopenia is of unfavourable significance; lymphocytosis the reverse Piney (1928) observes · "This [lymphocytopenia] is almost invariably of bad significance" (cf. Naegeli, 1931). And again. "The subsidence of an infection, and the period of convalescence are associated with changes of extreme importance. The neutrophiles gradually fall to normal numbers but the lymphocytes increase, both relatively and absolutely, to figures well above normal . . . it [lymphocytosis] is, therefore, an indication of an infective lesion which has been overcome or, at least, is being held in check."

In tuberculosis the favourable significance of lymphocytosis has been noted by many workers (Murphy, 1926) Smithburn (1932), on the other hand, found that "chick embryo extract given intravenously did not favourably influence the course of experimental tuberculosis in rabbits, although it did cause lymphocytosis and lymphoid hyperplasia". The lymphocytosis in Smithburn's experiments was not very marked, nor was it sustained throughout the experimental period. For a more recent review the paper by Medlar, Lotka and Spiegelman (1940) should be consulted.

Lymphocytes in Bone Marrow

Normal Marrow

In Chapter 6 it was pointed out that the disappearance of lymphocytes from the blood might be accounted for in one of four ways; (1) by senescence and degeneration while in the blood; (2) by passage into lymph; (3) by excretion into the lumen of the alimentary canal; and (4) by passage into the bone marrow. The first three of these possibilities have already been dealt with, and we shall now discuss the fourth, the possibility that lymphocytes are filtered out of the blood stream into the bone marrow. We are not concerned here with the occasional presence of organized lymphoid nodules in the marrow (see Chapter 6), but rather with the more regularly occurring and diffusely scattered small lymphocytes

Significance of Lymphocytes in Marrow: Quantitative Data

From what has been written so far it would appear that the marrow contains a surprisingly large number of small lymphocytes which reach it via the blood stream On the whole, the evidence appears to indicate that these can become transformed into blast cells and so function as the precursors of erythrocytes and granulocytes. But if this be so one would expect that marked changes in the rate of red or white cell formation should be reflected in changes in the marrow lymphocytes, and we now propose to discuss some of the evidence which can be brought to bear on this aspect of the problem. It is greatly to be desired that the evidence should not merely be qualitative, as is so frequently the case in the discussion of marrow changes, but also quantitative. Quantitative information about the various cell groups concerned can serve as a very healthy corrective of much speculation concerning theories of haemopoiesis.

For human marrow, unfortunately, little quantitative information is available. There are a few scattered observations such as those of Isaacs (1937) who used chiefly autopsy material and obtained counts of 900,000–1,000,000 per c.mm., Gordon (1939), who obtained (autopsy, accident) a count of 468,000 per c.mm, and Lossen (1910) who in a child shortly after birth obtained a count of 1,400,000 per c mm. It is probable that the absolute count of nucleated cells per c.mm. of human marrow is rather lower than that usually obtained in guinea-pigs, though more human data will be needed before this can be stated definitely. However, the percentage data for the different cell groups in human marrow are very extensive and, as far as the lymphocytes are concerned, we have already noted that most haematologists would regard 10 per cent as being the normal lymphocyte content in adult marrow, though in infancy the

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lymphocytes are much more numerous. Thus Doan and Zerfas (1927) in a child aged 4 years found the lymphocytes to be 37 per cent of the nucleated marrow cells. For our main quantitative data we shall rely on studies of guinea-pig marrow (Yoffey *et al.*, 1952, 1954, Harris *et al*, 1954; Yoffey, 1955; and much unpublished work).

The question at once arises to what extent results which have been obtained in one species may be held valid for others also. It seems difficult to believe that if the lymphocyte can function as a stem cell in the guinea-pig it would not function similarly in other animals. Yet there exist important species differences For example, one has only to look at rabbit and guinea-pig marrow after the injection of a vital dye to observe that the former contains many more phagocytic cells—both endothelial and otherwise—than the latter. Cappell (1929) makes a similar observation in comparing rabbit and mouse. If—as suggested by Ferrata (1918) and others—these phagocytic cells can function as stem cells, there might be less demand on the lymphocytes, and this might possibly have something to do with the species differences in lymphocyte output. It is also possible that the intermediate stages between lymphocytes and other cells might be more prolonged, in which case again the demand for lymphocytes might be less.

One of the fundamental problems about the marrow lymphocytes is whether they are myelogenous or haematogenous, and we have (p. 336) previously given the evidence in favour of the view that they are haematogenous. In the 400 gm. guinea-pig, using the thoracic duct data of Reinhardt and Yoffey (1955), the daily output of lymphocytes is 372×10^6, the number of lymphocytes present in the circulation is 131×10^6 and the number present in the bone marrow ranges from $1{,}200–2{,}400 \times 10^6$ In other words, for every lymphocyte normally present in the blood there are 9–18 present in the marrow, while 3 are daily entering via the thoracic duct It will be appreciated, in view of what has been written in Chapter 6, that many more lymphocytes may in fact be entering the blood than do so via the thoracic duct Furthermore there may, as has already been emphasized, be other regions in which lymphocytes can leave the blood—e g the intestinal submucosa which according to Kindred (1942) contains three times the number of lymphocytes present in the blood. But as far as we know, apart from the lymphoid tissues the bone marrow possesses the largest of all the extravascular collections of lymphocytes, more than sufficient for those which must continually be leaving the blood. From the point of view of cell replacement, the greatest need is for members of the erythroid series, and if lymphocytes can function as stem cells we should expect to find the most marked changes in marrow lymphocytes in relation to disturbances of erythropoiesis.

Experimental Approach

Haemorrhage. One of the most obvious lines of attack is the response of the lymphoid tissues and bone marrow to haemorrhage. This is the type of approach employed by Sjovall (1936), who argues that if it is true that lymphocytes can serve as stem cells for erythrocytes, then the production of experimental anaemia by repeated haemorrhage should result in an increased demand for these lymphocytic stem cells To meet this demand, the lymphoid tissues should undergo hypertrophy. In Sjovall's experiments this did not occur. On the contrary, the lymphoid tissues atrophied somewhat and in addition there was a fall in the level of the blood lymphocytes.

The theoretical basis of Sjovall's experiments seems quite reasonable and we think the experiments should be repeated, with special attention to some points which Sjovall disregarded. It has already been pointed out (Chapter 6) that the level of the blood lymphocytes is not a reliable index of lymphocyte output. Lymphocytes may be entering the blood in numbers which are normal or even greater than normal, but if they are leaving the blood in still greater numbers than those in which they are

animals rendered anaemic. In this connection the "second top" lymphocytes of Fichtelius (1953) obviously come to mind. It is also possible that, if the anaemia is too prolonged, atrophy of the lymphoid tissues may develop, either through exhaustion as suggested by Jordan (1936), or from the effects of the prolonged anaemia. It would be interesting to see what would happen in anaemias of varying duration.

Much more attention should be devoted to the bone marrow in experiments of this nature, since vital information should be derived from the quantitative and qualitative changes in the lymphocytes at various stages of anaemia. Sjovall performed a few differential counts, but mainly in sections, and it is not surprising that he found them of little value.

Leucocytosis promoting factors. In recent years attention has been paid to a number of factors which have been claimed to give rise to leucocytosis. Among these we may mention Menkin's "Leukocytosis Promoting Factor" (LPF), the Plasma Expulsion Factor of Steinberg and Martin (1950), and lymphokentric and myelokentric acids (Turner, Miller and Flint, 1953). Menkin described LPF in 1940; it appears to be a polypeptide attached to α-globulin.

Harris, Menkin and Yoffey (1956) have given intraperitoneal injections of LPF to guinea-pigs, and then 4 hours later noted the quantitative changes in the marrow and the peripheral blood. It was found that

after 4 hours the marrow neutrophiles had fallen from 468,000 to 346,000 per c.mm., the change involving mainly the mature and the band cells. At the same time the marrow lymphocytes showed a significant increase from 396,000 to 516,000 per c.mm. This unexpectedly large and rapid [illegible] must have entered from the blood.

But simple calculation serves to show that during the period in which the total marrow lymphocyte population increased by 954×10^6 the blood lymphocytes fell by the comparatively small number of 7×10^6 while the thoracic duct output, assuming that it was proceeding at its normal rate—we have not yet directly measured the effect of LPF upon the thoracic duct lymphocytes—would have amounted during the 4 hour period to a mere 62×10^6. The source of the large increase *in marrow* lymphocytes after LPF remains therefore somewhat of a mystery. Either there are large reserves of lymphocytes somewhere in the body, reserves which can rapidly be mobilized, *or else far* more lymphocytes are entering the blood than are obtaining access to it via the thoracic duct.

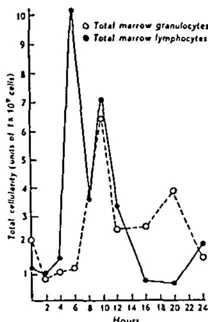


FIG. 87—The effect of a single intraperitoneal injection of staphylococcus aureas vaccine on the lymphocyte and neutrophile (pseudoeosinophile) content of bone marrow
(Redrawn from data of Ancill, 1955)

These experiments with LPF cover only a period of 4 hours. Ancill (1955) has performed experiments in which the marrow changes have been studied following the intraperitoneal injection of staphylococcal and other vaccines. Here again the first change, marked at 6 hours, is a significant increase in the marrow lymphocytes, which then diminish and fall to quite low levels at 20 hours (with an intervening peak at about 10 hours) as the granulocytes are being formed in increasing numbers (Fig 87). Quantitative experiments along these lines, if they can be confirmed and amplified, should help to clarify considerably the relationship between the lymphocytes and the other cells of the marrow.

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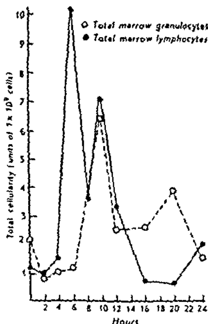


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their maturation once they reached it, so that they would gradually accumulate in the marrow in increasing numbers, and undergo no further development. Supply of the missing maturation factor, on the other hand, should result in a marked diminution in their numbers in the marrow.

Agranulocytosis. Apart from the neutropenia which follows the action of various toxic substances, the case for the existence of a primary maturation arrest of the granulocytes, the "pernicious leukopenia" of Fitz-Hugh and Krumbhaar (1932) seems at present to be fairly well substantiated. In this condition mature granulocytes are no longer discharged into the blood, *so that the blood granulocytes fall almost to zero. The relative percentage* of blood lymphocytes rises, but the absolute number usually falls, frequently to 200–300 per c.mm

The lymphocytes in the bone marrow are markedly increased (Aubertin and Levy, 1928, Nordenson, 1935, Jackson and Parker, 1935, Rohr, 1936, Vogel, Erf and Rosenthal, 1937, Rhoads and Miller, 1938 [we regard their "primitive cells" as lymphocytes], Rosenthal, 1938, Rohr, 1939; Braun, 1944, Blackburn, 1948) The accumulation of lymphocytes in the marrow may at times be so marked as to give a pseudo-follicular appearance (Custer, 1935, Rosenthal, 1938). Since the blood lymphocytes fall so sharply, we must assume either that large numbers of lymphocytes are being formed in the marrow, but not entering the blood, or that the marrow is taking from the blood all the lymphocytes it can. The latter seems to us the more probable explanation, since the follicular accumulations in the marrow do not seem to be true lymphoid nodules with cells showing active division.

If the marrow is taking up all the blood lymphocytes, one might explain this, as Custer (1935) does, on the grounds that one is dealing with "folliculoid accumulations in response to focal degeneration" But in the same paper Custer records a case of arsphenamine poisoning and a case of septic neutropenia, in which one might expect at least the same degree of "focal degeneration", yet hardly any lymphocytes were present in the marrow.

Agranulocytosis as met with clinically may have a varied aetiology, so perhaps a neutropenia of known origin is of more value for an analysis of the marrow changes. Some instructive figures are given by Blackburn (1947–1948) in an account of thiouracil neutropenia Marrow counts were performed in a patient on April 3rd, 5th, 7th and 10th, the last at a time when the blood granulocytes were beginning to show marked recovery Table 54 is based upon a patient reported by Blackburn (1947–1948), and in whom differential counts of sternal marrow were performed over a 7-day period, beginning at the time when the agranulocytosis was profound and ending when the granulocytes were beginning to reappear in the peripheral blood and the bone marrow was returning to normal. The

Steinberg and Martin (1950) described the presence in human plasma of an Expulsion Factor which, on injection into rabbits, induced a discharge of granulocytes from the marrow. Though they did not apply a quantitative technique to their study of the marrow, the percentage changes in the marrow indicate that 2 hours after the injection there was no obvious change, whereas 4 hours afterwards the cellularity of the marrow was markedly decreased, the mature granulocytes being mainly affected. By 15 hours the marrow had more than returned to normal, showing in fact hyperplasia with increase in the marrow granulocytes. Though they give no data on marrow lymphocytes, their experiments show the same speed of response by the marrow as was found by Harris, Menkin and Yoffey (1956).

The experiments of Harris, Menkin and Yoffey bring out one further quantitative relation of interest, namely a discharge from the marrow of 987×10^6 neutrophiles and a rise in the blood neutrophiles of 48×10^6. In other words, whereas the discharge of neutrophiles from the marrow should have given rise to a leucocytosis of around 32,000 per c.mm., the observed rise was 1,600 per c mm We cannot as yet account for the missing neutrophiles, but it is evident that only by a quantitative study of the marrow could the fact that they were missing have been brought to light The findings certainly fit in well with the idea of a rapid sequestration of granulocytes from the blood (Bierman, 1955, Osgood, 1954)

Ablation of bone marrow The removal of a substantial amount of bone marrow should, if the marrow is the main destination of the blood lymphocytes, result in an accumulation of these cells in the blood. Steinberg and Martin (1946) describe a technique for removing marrow from rabbit long bones and replacing with an inert material They estimated that they could ablate in this way as much as 41 ± 5 per cent of the total marrow. In 6 animals in which they rapidly removed this amount of marrow, the blood lymphocytes, which in the week before operation had ranged from 5,080 to 5,700 per c mm., averaged at 1 day after operation 4,590, at 3 days 13,340, at 5 days 15,800, and at 20 days 5,480 A result of this kind is certainly most intriguing and should well merit further investigation.

Marrow in conditions of Maturation Arrest

If it is true that many lymphocytes are constantly leaving the blood to enter the marrow and there develop into granulocytes and erythrocytes, one should be able to predict the occurrence of definite changes in conditions of maturation arrest. Such an arrest, presumably, would not interfere with the entry of lymphocytes into the marrow, but only with

their maturation once they reached it, so that they would gradually accumulate in the *marrow in* increasing numbers, and undergo no further development. Supply of the *missing maturation factor, on the other hand,* should result in a marked diminution in *their numbers in the marrow*

Agranulocytosis. Apart from the neutropenia which follows the action of various toxic substances, the case for the existence of a primary maturation arrest of the granulocytes, the "pernicious leukopenia" of Fitz-Hugh and Krumbhaar (1932) seems at present to be fairly well substantiated. In this condition mature granulocytes are no longer discharged into the blood, so that the blood granulocytes fall almost to zero The relative percentage of blood lymphocytes rises, but the absolute number usually falls, frequently to 200–300 per c.mm.

The lymphocytes *in the bone marrow are markedly increased* (Aubertin and Levy, 1928; Nordenson, 1935; Jackson and Parker, 1935, Rohr, 1936; Vogel, Erf and Rosenthal, 1937; Rhoads and Miller, 1938 [we regard their "primitive cells" as lymphocytes], Rosenthal, 1938, Rohr, 1939; Braun, 1944; Blackburn, 1948). The accumulation of lymphocytes in the marrow *may at times* be so marked as to give a pseudo-follicular appearance (Custer, 1935; Rosenthal, 1938). Since the blood lymphocytes fall so sharply, we must assume either that large numbers of lymp [illegible] tering the blood, [illegible] mphocytes it can. [illegible], since the follicular accumulations in the marrow do not seem to be true lymphoid nodules with cells showing active division.

If the *marrow is taking up all the blood lymphocytes, one might explain* this, as Custer (1935) does, on the grounds that one is dealing with "folliculoid accumulations in response to focal degeneration" But in the same paper Custer records a case of arsphenamine poisoning and a case of septic neutropenia, in which one might expect at least the same degree of "focal degeneration", yet hardly any lymphocytes were present in the marrow.

Agranulocytosis as met with clinically may have a varied aetiology, so perhaps a neutropenia of known origin is of more value for an analysis of the marrow changes. Some *instructive figures are given by* Blackburn (1947–1948) in an account of thiouracil neutropenia. Marrow counts were performed in a patient on April 3rd, 5th, 7th and 10th, the last at a time when the blood granulocytes were beginning to show marked recovery Table 54 is based upon a patient reported by Blackburn (1947–1948), and in whom differential counts of sternal marrow were performed over

is pro-

TABLE 54

Changes in the percentage composition of the bone marrow in a case of thiouracil neutropenia

Cells per cent	*April 3rd*	*April 5th*	*April 7th*	*April 10th*
Myeloblasts	2·5	2·4	0·9	0·5
Promyelocytes	3·0	1·5	5·0	4·2
Neutr. myelocytes	0·6	1·6	8·3	19·6
Neutr. metamyelocytes	0·1	0·6	2·9	21·3
Neutr. segmented	0·1	—	0·1	6·5
Total neutrophil	6·3	6·1	17·2	52·1
Lymphocytes	23·6	34·2	42·1	23·4
Bare nuclei	20·5	24·1	14·6	10·5
Reticulum	1·0	0·8	0·1	0·6

Note that neither on April 7th nor on April 10th, when granulopoiesis was progressing vigorously, were many mitoses observed either in the promyelocytes or the myelocytes (1 of each per 1,000 cells).

Data from Blackburn (1947-1948)

piling up of lymphocytes in the marrow (23·6 per cent on April 3rd, 34·2 per cent on April 5th, and 42·1 per cent on April 7th) during the period of agranulocytosis is very suggestive, as also is their fall to 23·4 per cent on April 10th, when recovery was under way. When expressed merely as a percentage, it is difficult to appreciate fully the significance of the fall in the marrow lymphocytes from 42·1 per cent to 23·4 per cent. But if we attempt to translate it into quantitative terms, a simple calculation on the basis of our guinea-pig data makes it evident that if this fall is due to lymphocytes being discharged into the blood, there should ensue a lymphocytosis of the order of 70,000–80,000 per c mm. Since no such lymphocytosis occurs, the only way in which the facts could fit in with the hypothesis that lymphocytes are passing from marrow to blood would be to assume either that they were rapidly destroyed in the circulation, for which we have no evidence, or that they were being removed from it in some region or regions unknown. But the evidence we have previously quoted shows that stimulation of granulopoiesis is in fact associated initially with a rapid increase in the uptake of lymphocytes by the marrow, and only at a later stage, when the granulocytes begin to be formed and discharged in greatly increased numbers, do the lymphocytes in the marrow diminish.

Pernicious anaemia. In the earlier literature, frequent reference is made to the appearance of "lymphoid" cells in the bone marrow in pernicious anaemia (e.g. Cohnheim, 1876). Ellermann (1920) noted numerous small lymphocytes scattered through the marrow, but no lymphoid nodules, such as had previously been described by Ziegler (1910). Ellermann found in addition large numbers of "lymphoid"

marrow cells which were, however, quite different from lymphocytes or lymphoblasts, and had a mitotic angle of 18°, as compared with 21° for the megaloblasts and 67° for neutrophilic myelocytes.

Dameshek and Valentine (1937) give a review of the literature, and also present results of their own. These investigators found that before liver treatment the lymphocytes on the average were 27 per cent of the myeloid cells, after liver treatment, 15·4 per cent "A striking finding in the marrow of the patient with pernicious anaemia is the presence of large numbers of typical lymphocytes having the characteristic size, shape and staining reactions of the mature, or small, lymphocytes of the peripheral blood . . . In relapse, these cells comprised about 25 per cent of the total number of white cells, with remission after continued therapy they became definitely diminished, in two cases none being seen. Peculiarly enough, with these large numbers of cells, transitions from a more immature type of lymphocyte were not seen. This, together with the lack of clustering of these cells in groups or islands, suggests an infiltration by mature lymphocytes rather than a proliferation of lymphoblastic cells." In other words, it suggests the entry of large numbers of lymphocytes from the blood into the marrow On the other hand, the disappearance of lymphocytes from the bone marrow is one of the striking and almost incredibly rapid effects of liver extract The loss of lymphocytes from the marrow could of course be interpreted as a rapid discharge into the blood to make way for proliferating red cells But if as before we make calculations on the basis of the quantitative data available in the guinea-pig, the fall in the marrow lymphocytes should result in a lymphocytosis of something like 40,000 per c.mm.; and this does not occur

Aplastic anaemia In primary, or idiopathic, aplastic anaemia there are to be found two types of bone marrow, one of which is aplastic the other hyperplastic Holmes and Broun (1932–1933) found in a case of aplastic anaemia that the lymphocytes constituted 42 per cent of all nucleated marrow cells. Rhoads and Miller (1938), in 15 hyperplastic marrows in cases of aplastic anaemia, found an average count of 36·9 per cent of primitive cells, while in only 2 out of the 15 cases did they note the presence of lymphocytes They conclude that these primitive cells are not lymphocytes, but from what we have previously said concerning the relation of these two cells, and also from an analysis of the pernicious anaemia data of Rhoads and Miller, in which their primitive cells correspond to the lymphocytes of other workers, it is evident that their primitive cell and lymphocyte may be regarded as identical. This is further borne out by the fact that in a case of agranulocytosis Rhoads and Miller found that 57 per cent of the marrow cells were primitive. In a later paper Bomford and Rhoads (1941) conclude that many of the cells which Rhoads and Miller had previously considered to be "primitive" cells are in fact early

erythroblasts. Davidson, Davis and Innes (1944) note in cases of refractory anaemia the appearance of what they term Q cells, which from their description seem very like enlarging transitional lymphocytes.

While the etiology of aplastic (or "refractory") anaemia is unknown, and while undoubtedly the name, as clinically employed, covers a number of fundamentally different conditions, it nevertheless seems likely that if we exclude cases in which there is an obvious contributory factor—such as osteosclerosis or the action of some toxic agent—there remains a group of patients in whom there is no marrow aplasia, but in whom there is, nevertheless, a marked diminution of all the blood cells (the "pluricytopenia" of Rhoads and Miller). Rhoads and Miller conclude that in cases such as these the basic change is "a failure of maturation of hemopoietic cells at an early undifferentiated stage. Comparison of marrow from persons presenting aplastic anemia with marrows from persons presenting acute agranulocytosis shows in the latter a similar lack of maturation and suggests that aplastic anemia and acute agranulocytosis may have some etiologic factor in common." Jordan (1939) interprets the basic change as an accumulation in the marrow of small lymphocytes, which do not undergo further development Our first quantitative approaches to the study of bone marrow and its reactions seem to fit in best with this latter view This interpretation appears to be reinforced by the observation that the trend can be reversed, and the lymphocyte population of the marrow can rapidly diminish when there is augmented erythropoiesis or granulopoiesis.

The hypothesis which best fits in with all the facts presented is that the small lymphocyte is a specialized form of the primitive mesenchymal cell, in a resting, relatively inactive state and reduced to the smallest possible size for the purpose of easy mobilization and transport through the blood-stream. In response to stimuli as yet unknown it can once again revert to an active state and undergo development into other cell types.

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cuff was gradually inflated (see Table 5, p. 73). In the acute phase of oedema formation arising from venous obstruction the sudden increased filtration from the capillaries raises the tissue tension, the lymphatics dilate and the lymph flow greatly increases.

In man, McMaster (1937*a*) has observed the flow of lymph in the

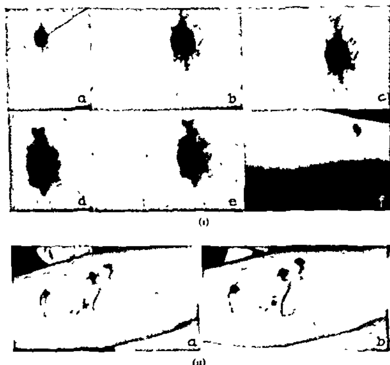


FIG 88

(i) a–e Photographs of the distribution of dye on intradermal injection into the skin of the volar surface of the forearm The photographs were taken 30 and 45 seconds and 1 2 3 and 20 minutes after beginning the injection f is reduced in size to show a coloured streamer extending up the arm from the injected area. (ii) Streamers from intradermal dye injection, 1½ and 3 minutes after release of circulatory obstruction. The circulation had been obstructed for 40 minutes

(From McMaster 1942)

cutaneous vessels by injecting intradermally very small amounts (0 02 ml.) of dye, under approximately the same pressure as that existing in the tissues In this way the injected fluid is not forced along the lymphatics by the injection, but clear, normal lymph on passing through the area carries away some of the dye. Blue streamers are therefore formed in the limb, Fig. 88(i), which are a measure of the rate of flow along the large lymphatic channels. If the venous return of the limb is obstructed by a cuff, no flow is observed, but as soon as the cuff is released long

CHAPTER 8

PRACTICAL CONSIDERATIONS

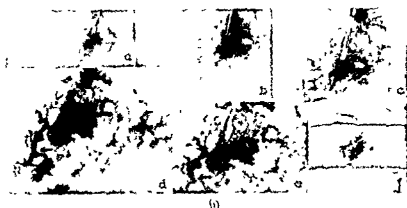
In each of the previous chapters we have discussed many applications of the lymphatic system to clinical disorders. It is not our purpose here to repeat these practical considerations, but to discuss in more detail some of the disorders of function involving lymphatics which have so far been dealt with either briefly or not at all.

Circulatory Oedema

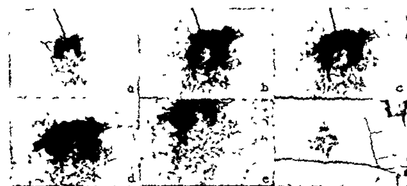
From our discussion of the formation of lymph (Chapter 2), we should expect the lymphatics to play a prominent rôle in restoring the fluid balance in an oedematous tissue. Oedema resulting from circulatory difficulties is due principally to raised venous pressure with a consequent capillary hypertension. The balance between the capillary pressure and the osmotic pressure of the plasma proteins is displaced in favour of the former, so that capillary filtration is increased. This continuous outward filtration results in the formation of excess tissue fluid or oedema, a process which is arrested ultimately by the rising tissue pressure.

The oedema may be localized to the region of an obstructed vein, such as may occur from venous thrombosis or from pressure exerted by a tumour. The lymph flow in these clinical conditions in man has not been measured; but, experimentally, in animals it has been shown that venous obstruction in a limb rapidly leads to a considerable increase in flow. Cohnheim (1889) quoting the work of Emminghaus writes, "On introducing a cannula into one of the lymphatics in the outer side of the leg of a dog, an extremely small quantity of lymph flows off from the foot, scarcely a drop in several minutes, as long as the limb is at rest. On now ligaturing the principal veins conducting the blood out of the leg, or on surrounding the thigh just above the knee with a ligature which must not be drawn too tightly, the lymph at once begins to drop from the cannula, so that you can now obtain in the same time as many (and indeed more) cubic centimetres of lymph as you formerly obtained drops While the increase in the lymph stream sets in immediately on applying the ligature, a tumefaction of the foot is only slowly and gradually developed during the space of several hours." These experiments have been elaborated and the findings confirmed by Field and Drinker (1931) and White, Field and Drinker (1933) who placed a cuff around the limb of a dog and showed that the lymph flow increased progressively as the

all day but readily become normal on resting, much of the oedema fluid will be reabsorbed into the blood capillaries when the capillary pressure is reduced, but the lymphatics must also be effective in returning to the blood stream the protein that has become extravascular. McMaster



(i)



(ii)

Fig. 89

(From McMaster, 1942)

showed the effect of posture on lymph flow in a normal limb. In a subject seated quietly with the feet resting on the floor, streamer formation following intradermal injection of dye was absent. On elevating the leg, however, streamer formation was rapid. Similar changes in lymph flow probably occur in oedema of the legs in the early stages of cardiac decompensation

streamers rapidly form indicating that the lymph flow is greatly increased, Fig. 88(ii) In man, as in animals, therefore, localized venous obstruction will give rise to an increased lymph flow.

Generalized circulatory oedema arises in cases of heart failure. The oedema fluid may accumulate either in the regions drained by the venae cavae, as a result of weakness of the right heart, or in the lungs usually from left heart failure. The effect on oedema formation of increased venous and, therefore, capillary pressure, is strikingly seen in the early distribution of the oedema. In the ambulatory patient, the ankles may swell during the day and go down at night, while in the bed-ridden patient oedema is most prominent in the sacral region The increase in capillary pressure due to gravity is evident in these observations.

The oedema fluid contains protein which is probably somewhat less than the protein concentration in normal tissue fluid because the filtering head of pressure is increased. Fishberg (1942) quotes average figures of 0 35 (generally 0 2 to 0·5) per cent for the subcutaneous, 2·11 per cent for pleural, 2·34 per cent for ascitic, 3 08 per cent for pericardial and 2 to 3 per cent for alveolar fluid These figures are comparable with values obtained in normal animals for tissue fluid or lymph from these regions (Chapter 3). It is, therefore, difficult to say whether in heart failure the lowered oxygen tension in the tissues increases the capillary permeability. At least, there is no gross increase in permeability such as we see in acute inflammation.

What concerns us here, however, is not so much the causes of circulatory oedema (cf. Fishberg, 1942; Davis and Smith, 1947; Richards, 1949), but the rôle of the lymphatics in restoring the normal fluid balance. In this, as well as in all other types of oedema, the lymphatic vessels are widely dilated. This is not due to any blockage of the lymphatic channels, but, as we have observed in Chapter 1, because of the lymphatic attachments to the surrounding tissues. Although there is no obstruction in the course of the lymph vessels, the raised venous pressure may to some degree hinder the flow of lymph into the veins at the base of the neck. McMaster (1941–1942), however, gave evidence to show that this did not greatly affect the flow of lymph in limb lymphatics.

We have no direct quantitative information concerning the volume of fluid returned to the blood stream by the lymphatics in cardiac oedema in man. From our description of the regional lymphatics in Chapter 3, we should expect that any fluid that leaks into the peritoneal or pleural cavities would be fairly rapidly removed by the lymphatics of those regions. Fluid in the pulmonary alveoli or in the skin and subcutaneous regions, however, should be removed much more slowly by the lymphatics; but the lymphatics are, nevertheless, important in restoring the fluid balance in these tissues. In those mild cases in which the feet swell after standing

theories advanced to explain the changes in fluid balance is entirely ... There are probably several causes for the increase of extra-

...

cause of this increase in extracellular fluid. A fall in plasma protein, especially of the albumin fraction, usually occurs in undernutrition and the consequent decrease in the colloid osmotic pressure of the plasma augments capillary filtration and so precipitates visible oedema. Oedema may occur, however, in cases in which the plasma proteins are within normal limits. In experimental undernutrition in dogs, Weech and his

...

... Weech, 1938–1939). They found that, on a ...

change was observed in the globulin. Another factor in precipitating oedema in malnutrition is the salt intake; as in circulatory oedema, a ... of salt will increase the volume of fluid in the extracellular

...

The protein content of the subcutaneous fluid is always low. ... (1951) quotes figures of 0·2 to 0·3 per cent while Weech (1938–1939) gives values of 0·1 to 0·6 per cent in man. The oedema fluid in nephrosis, another condition in which the serum albumin is low, also contains but little protein, usually less than 0·1 per cent. In dogs the protein level varied in subcutaneous oedema fluid from 0·02 to 0·72 per cent with an average of 0·23 and in ascitic fluid ... cent with an average of 0·18. Weech, Goettsch a... low protein level in the tissue fluid ... to a decreased permeability of the capillaries to protein. It would seem more likely, however, to be due to the increased filtration of plasma with a low protein concentration. The tissue fluid will ultimately resemble capillary filtrate. Since the increased filtering head of pressure is attained, not by raising the capillary pressure but by lowering the plasma albumin concentration, the level of proteins in the capillary filtrate and therefore in the tissue fluid will be somewhat lower than in cardiac oedema.

There are no direct measurements of the flow of lymph in hunger oedema in man. In cases in which the skin is lax, it might reasonably be postulated that the tissue tension would not rise so readily as in well nourished individuals and that the lymph flow would consequently not increase. In dogs Weech, Goettsch and Reeves (1934) collected lymph from legs which were oedematous as a result of malnutrition (low protein diet) or of chronic plasmaphaeresis. The protein content of the oedema

In cases of chronic cardiac oedema in elderly patients, on the other hand, intradermal injection of dye did not lead to streamer formation (McMaster, 1937*b*). The lymphatics of the skin were widely dilated, but they were full of stagnant lymph, Fig. 89. "No matter how much the lymphatic channels were dilated in cases of cardiac edema, we never observed the formation of coloured streamers. There was no evidence of lymph flow, yet the fact that the lymphatics were patent could readily be demonstrated. When a region stained as the result of an intradermal injection of dye was massaged, coloured streamers promptly appeared. If the skin of the lower leg of a patient with long standing edema was stroked from the injection site toward the periphery, a retrograde passage of dye took place along the superficial lymphatics The phenomenon was never seen in normal man, nor did it occur in the patient a few days after the edema had been reduced by therapeutic measures. It is plainly *indicative of a valvular incompetence of the lymphatics*" (McMaster, 1941–1942). It would seem, therefore, that in grossly oedematous limbs, the valves of the lymphatics become incompetent, so that if the tissue tension is raised by movement, lymph can pass towards the periphery just as readily as it can centrally. In such circumstances the lymphatics probably do not play a prominent rôle in restoring the fluid balance of the limb, until the valves become competent. With a fall in capillary pressure following treatment much of the subcutaneous oedema fluid will be reabsorbed into the blood capillaries, but the lymphatics must then return the extravascular protein to the blood stream before the normal fluid balance is restored.

In contrast to the stagnation of lymph in the oedematous legs of cardiac patients, McMaster found an increased flow in the skin of patients with oedema accompanying nephritis. This was especially evident at the onset of diuresis. The lymphatics were not so widely dilated as in the patients with cardiac oedema and the lymph flow suggests that the valves were not incompetent. McMaster concluded from these experiments that "in heart disease the lymphatics fail in their function of fluid transport, adding to the edema. In nephritis on the other hand the lymphatics aid in the removal of the accumulated fluid." It is doubtful, however, whether this generalization would apply to all stages of cardiac and nephritic oedema, or to lymph flow along the deeper lymph channels of the limbs which cannot be observed by the methods employed by McMaster.

Hunger Oedema

Oedema has been known throughout recorded history to occur during famines or prolonged malnutrition. McCance (1951), in an excellent review of the aetiology of hunger oedema, concludes that none of the many

and Barnard, 1931; Nix *et al.*, 1951*a* and *b*; Gray, 1951). Whether this ascitic fluid comes directly from the lymphatics in the capsule or merely is some of the excess tissue fluid which reaches the capsule actually outside the lymphatic vessels, the end result is the same. Once in the peritoneal cavity as free fluid, the drainage will be mainly through the lymphatics of the diaphragm (see p 176) Ascites will result, therefore, when the production of tissue fluid in the liver is so great that the liver lymphatics cannot cope with it, and the "spillover" is so great that the diaphragmatic lymphatics cannot remove it quickly enough

It is clear, therefore, that based on experimental evidence in animals, we cannot expect portal hypertension alone to produce gross ascites. Back pressure on the inferior vena cava above the diaphragm as in heart failure, constrictive pericarditis or thrombosis of the hepatic veins, or cirrhosis of the liver will lead to a raised pressure in the liver sinuses and an increased filtration through the walls of the sinuses Excess fluid accumulates in the spaces between the sinus wall and the liver cells, and tracks to the surface of the liver lobule where it is absorbed into the liver lymphatics (see p 142) Ascites does not necessarily ensue It is only in severe cases of raised pressure in the hepatic vessels that the filtration rate is increased sufficiently to produce ascites.

In experimental obstruction of the inferior vena cava or of fibrosis of the liver, gross ascites may be precipitated by lowering the plasma protein concentration or by increasing the sodium retention in the body (McKee *et al.*, 1948) Similar possible mechanisms in precipitating gross ascites in a patient with cirrhosis of the liver have been discussed by Hyatt and Smith (1954).

We see, therefore, that in the shifts of fluid which take place when ascites develops, three major sets of lymphatics are involved—the lymphatics of the alimentary tract, the liver lymphatics and the lymphatics of the diaphragm. All three, as we have seen in earlier chapters, are capable of carrying very large volumes of lymph, much greater than the lymphatics from any other region of the body It is, therefore, only in extreme circumstances and in the presence of severe disease that gross ascites becomes evident These facts also account, in part, for the difficulty of producing regularly gross ascites in the experimental animal.

Reaction to Injury

Many of the functions of the lymphatic apparatus are displayed prominently in the reactions of a tissue to injury. A local accumulation

fluid in both groups of dogs was of the same order of magnitude as obtains in man with hunger oedema. The lymph flow was determined in animals under nembutal anaesthesia and in unanaesthetized animals. Lymph was obtained from the anaesthetized group by massage but in the unanaesthetized group the flow was spontaneous on walking. The flow from the leg ducts of normal dogs is very variable which makes a comparison with that of oedematous animals difficult. On the whole, however, the lymph flow from the oedematous limbs was shown to be slightly greater than the flow in normal animals. It would seem, therefore, that in these conditions the lymphatics are functioning to alleviate the oedema. When the serum proteins were raised by feeding protein, the oedema disappeared. With the rise in colloid osmotic pressure most of the oedema fluid would be absorbed into the blood vessels more rapidly than it could be removed by the lymphatics. Any excess extravascular protein, however, would be taken up by the lymphatics.

Ascites

In discussing the functional significance of the lymphatic drainage of the liver (p 148) and of the peritoneal cavity (p. 188), we have mentioned the rôle of the lymphatic vessels not only in absorbing the ascitic fluid but also in producing this fluid. There seems no doubt that the absorption of ascitic fluid through the diaphragmatic lymphatics is fairly rapid even in human beings The production of excess tissue fluid in this region must, therefore, be greatly increased before the signs of gross ascites are manifest The question that has often been asked, and which physiologists have attempted to answer, is " Where is the ascitic fluid produced ? "

Gross ascites in animals similar to that observed in cirrhosis of the liver in man is not easy to produce experimentally. Partial occlusion of the portal vein leads to an increase in capillary pressure and of capillary filtration in the wall of the alimentary tract. The excess tissue fluid formed will result in a great increase in the lymph flow from the thoracic duct (Starling, 1894), a transudation of fluid into the lumen of the gut (cf. Verzar and McDougall, 1936), but only a transient ascites (Volwiler, Grindlay and Bollman, 1950 , Schilling *et al.*, 1952). When, however, the hepatic vein or the inferior vena cava just above the diaphragm is partially occluded, gross ascites may ultimately be produced (cf. Bolton, 1909, 1914 ; McKee *et al.*, 1948 ; Nix *et al.*, 1951). Also, experimental cirrhosis of the liver, caused by repeated administration of carbon tetrachloride may produce ascites (Nix *et al.*, 1951*a* and *b*). It would appear, therefore, that in experimental ascites most of the ascitic fluid comes from the liver. In these cases the lymphatics of the liver, which drain into

the capillary membrane with a resultant intense oedema of the injured skin, Fig. 90 (Courtice, 1946). Whereas normally the globulins escape from the capillaries less readily than the albumin, after a thermal burn the protein molecules all pass through the capillary wall at about the same rate as the smaller molecules (Perlmann, Glenn and Kaufman, 1943; Cope and Moore, 1944). If, however, the temperature is excessively high, the tissues, including the blood vessels, will be coagulated or destroyed. In these circumstances no blood will flow through the area. For example, if a rabbit's ear is placed in water at 100° C for half a minute, the whole ear will shrivel up with little or no oedema formation, whereas if the ear is placed in water at 65° C for the same time, it will ultimately swell enormously

In any injury, therefore, there will be zones in which the capillaries are affected in different ways—the zone in which the capillaries are necrosed or thrombosed and through which blood no longer flows, the zone in which the capillary membrane loses its effectiveness as a barrier to protein and the zone in which the capillaries may be altered in calibre but are otherwise normal. There may also be similar grades of injury to the cells of the injured tissues (Leach, Peters and Rossiter, 1943) The extent of each of these zones in an injury largely determines the extent of protein extravasation. In some instances, the first zone of tissue necrosis may be small, yet surrounded by a large zone of intense oedema. Such is the case with skin contamination of certain vesicant liquids (Cameron, Courtice and Short, 1947, Cameron and Courtice, 1948), and in mechanical injuries such as the experimental crush syndrome (Bywaters and Popjak, 1942, Swingle *et al*, 1942, Duncan and Blalock, 1942)

Lymph flow in acute phase

Since the extravascular fluid in the injured tissues is rich in protein, it is returned to the blood stream mainly by the lymphatics Experiment has shown that soon after injury the lymph flow increases considerably as the pressure of the interstitial fluid rises, and the protein concentration of the lymph rises until it approximates to that of the plasma Field, Drinker and White (1932) found lymph pressures in the leg lymphatics of anaesthetized dogs as high as 120 cm of lymph after severely scalding the foot. If the lymph is being collected from the limb of an anaesthetized animal, there will be no spontaneous flow before injury, but after injury the rise in tissue tension may be so great (as observed by Field, Drinker and White) that lymph flows spontaneously, i e lymph will flow without movement of the limb Passive movement of the limb will further increase the flow The lymphatics, therefore, are capable of increasing the protein absorption many times above normal in the early stages of an acute inflammatory reaction

of fluid in the extravascular spaces, leading to swelling of the damaged tissues, follows all injuries. The nature of the fluid loss depends upon the type and intensity of the injury. In mechanical trauma the tissues may be crushed and lacerated with widespread rupture of the blood vessels, resulting in an effusion of blood into the neighbouring extravascular spaces. The blood vessels of less severely damaged tissue may not be ruptured, but the permeability of the capillary membrane to the plasma proteins increased. The swelling of the damaged tissue in mechanical trauma is, therefore, due in part to haemorrhage, but in part also to the effusion of a protein-rich fluid resembling plasma (Parsons and Phemister, 1930; Blalock, 1930, 1931*a*, *b* and *c*; Freedlander and Lenhart, 1932). In those injuries such as thermal or chemical burns in which there is little or no rupture of the blood vessels, intense local oedema is due almost entirely to exudation of a protein-rich fluid through the damaged capillary walls (cf. Menkin, 1938). The protein concentration of this fluid is always high, approximating to that of plasma (Beard and Blalock, 1931; Harkins, 1945; Cameron and Courtice, 1946).

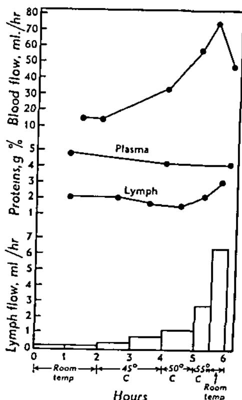


FIG 90—The effect of local temperature on the protein concentration and flow of lymph and of blood from the fore-paw of a dog

(From Courtice, 1946)

Since tissue fluid and lymph from any tissue are of approximately the same composition, the effects of various grades of injury on the protein concentration of the tissue fluid can be measured by collecting lymph from the affected region. When the temperature of a limb is raised by immersing it in water at 37 or 45° C., the arterioles dilate, so increasing the capillary pressure and blood flow through the region This results in an increased formation of capillary filtrate and of tissue fluid, but with no significant alteration in capillary permeability to protein until the temperature exceeds 50° C. At about 55° C, however, the permeability alters to allow easy passage of the protein molecules through

the capillary membrane with a resultant intense oedema of the injured skin, Fig. 90 (Courtice, 1946). Whereas normally the globulins escape from the capillaries less readily than the albumin, after a thermal burn the protein molecules all pass through the capillary wall at about the same rate as the smaller molecules (Perlmann, Glenn and Kaufman, 1943; Cope ar

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Not only protein but also extravasated red cells are absorbed by the lymphatics. If whole blood has escaped into the injured area the lymph will become blood-stained as the red cells are absorbed (McMaster and Hudack, 1934; Katzenstein, Mylon and Winternitz, 1943) The amount of extravasated blood absorbed into the lymphatics will depend not only on the degree of the haemorrhage, but on the tissue involved and the rapidity with which clotting occurs. If blood escapes into the peritoneal cavity, for example, the red cells will fairly rapidly be absorbed provided the blood does not clot; on the other hand lymphatic absorption of red cells from the injured tissues of a limb will be slow and coagulation will further reduce the chances of many of the red cells being returned to the blood stream intact

The question of the absorption of toxic substances liberated from the damaged cells of the injured area has received much attention in the study of experimental shock (cf. Harkins, 1941). If any of these substances were protein in nature, we should expect them to be absorbed mainly by the lymphatic vessels. Leach, Peters and Rossiter (1943) have postulated that in the moderately damaged area, sufficient injury to the tissues may have been done to damage cell permeability without altering the nature of some of the enzymes These enzymes may then diffuse out and prove to be toxic in parts of the body in which they are foreign. Kellaway and Rawlinson (1944) showed that in perfused limbs alkaline phosphatase, lipase and proteolytic enzymes were set free at temperatures above 41° C If these enzymes, which are protein in nature, are free in the tissue spaces it would seem likely that they would to some extent enter the lymphatics, whereas the liberation of smaller molecules, such as histamine, into the tissue fluid would not necessarily involve the lymphatics in the transport of such substances to the blood stream Direct evidence that the lymph from an injured area contains a foreign protein is not conclusive. Perlmann, Glenn and Kaufman (1943) examined electrophoretically the lymph from the forelegs of calves before and after severely scalding the legs. They found a new component in the electrophoretic pattern of the lymph collected after the burn and suggested that this was probably a tissue protein released by cellular destruction. Katzenstein, Mylon and Winternitz (1943) collected thoracic duct lymph from dogs after the release of tourniquets to the hind legs; they then injected this lymph intravenously into other dogs and in 50 per cent of cases observed a considerable fall in blood pressure. These experiments, therefore, suggest that, though the lymph from an injured region consists mainly of fluid which has escaped from the circulating blood, other substances released into the tissue fluid by the destruction of cells may also be absorbed by the lymphatics.

The lymphatics of the oedematous region of an injured tissue will

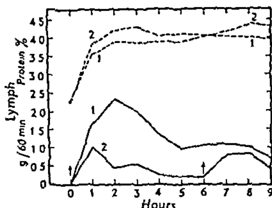


FIG 91 —Lymph flow and lymph protein concentration from the burned front paws in an anaesthetized dog At the first arrow both feet were burned for 30 seconds in water at 100° C

Curves 1 and 1 are from the right front paw which received no treatment and curves 2 and 2 are from the left front paw which was put in a plaster cast immediately after the injury At the second arrow the cast was removed

(From Glenn, Gilbert and Drinker 1943)

therefore be widely dilated and the flow of lymph in them will be greatly increased in the early stages The actual increase in flow will depend, among other things, on the actual rate of formation of tissue fluid If

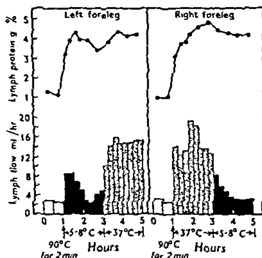


FIG 92 —The effect of local temperature on lymph flow from the fore-paw of a dog after a thermal injury

Immediately after immersing both fore-paws in water at 90° C for 2 minutes, the left fore-paw was kept in a water-bath at 5-8° C while the right fore-paw was kept at 37° C After two hours the water-baths were changed Cooling the paw greatly decreased the lymph flow while warming increased it.

(From Courtice, 1946)

after an injury this is decreased by applying pressure bandages (Barnes and Trueta, 1941; Glenn, Gilbert and Drinker, 1943) or by keeping the injured region cold (Courtice, 1946) the lymph flow will not be as great, Figs. 91 and 92. Later, when the extravasated fluid clots, the

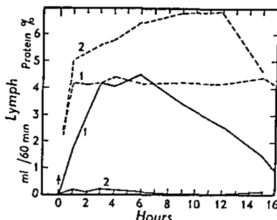


FIG 93.—The effect on lymph flow and protein content of immersing the front feet of a dog in water at 100° C for 2 minutes at arrow o Curves 1 and 1 for the right front foot show the lymph flow and protein content of the lymph for 16 hours after the burn Curves 2 and 2 are for the left front foot into which tissue extract was injected subcutaneously immediately after the burn The foot swelled enormously but the lymph flow was negligible since the tissue extract caused coagulation of the oedema fluid

(From Glenn, Peterson and Drinker, 1942)

lymph flow decreases A sudden decrease in lymph flow from an injured area in the acute phase may be brought about by injecting a tissue extract which coagulates the oedema fluid, Fig 93 (Glenn, Peterson and Drinker, 1942). Another factor in the falling off of the lymph flow after a time is probably a fall in tissue tension due to a loss of elasticity of the stretched tissues

Recovery phase of an injury

Absorption of fluid. When the blood capillaries regain their normal permeability, one of the problems in the complete restoration of the fluid distribution in the injured tissue is the reabsorption of all the extravasated protein and of the fibrin clot While the fibrin clot and the entangled red cells are broken down to smaller molecules, there is some evidence that the albumin and globulin fractions are absorbed by the lymphatics as such without enzymic degradation (Courtice and Simmonds, 1949) This lymphatic absorption in the later stages of recovery is, however, much slower than in the acute phase, partly because of the clotting of the fluid and partly because the tissue tension is not as great as in the initial phase when the connective tissues are suddenly stretched

Regeneration of lymphatics in healing wounds. Complete restoration

of the fluid equilibrium will also depend in part on the regeneration of lymphatics in the injured area. We have already observed in Chapter 1 that the lymphatic endothelium, like the vascular endothelium, has remarkable powers of regeneration and proliferation. Thus regeneration of the lymphatic vessels in the healing of a wound has been investigated in several preparations.

The simplest injury to study in this way is an aseptic skin incision Clark (1922–1923) observed that severed lymphatics in the tadpole's tail ultimately joined up again by the process of sprouting. In mammals Reichert (1926) made a complete section through the thigh of the dog with the exception of the femur, femoral artery and vein. All the lymphatics of the thigh were, therefore, severed He then sutured the cut tissues together and examined the lymphatics some time later by injecting India ink into the foot-pad and skin of the leg and thigh to show them up. In those cases where the tissues were handled very gently, the cut surfaces accurately approximated, the haemorrhage reduced to a minimum, and where strict asepsis was observed, regeneration was seen in the superficial lymphatics as early as 4 days and in the deep lymphatics 8 days after operation. Where there was any appreciable scar or where healing had been interfered with by trauma, foreign body or infection, the growth of lymphatics across the incised region was delayed for weeks or months.

McMaster and Hudack (1934) observed the behaviour of the lymphatics of the mouse's ear after a skin incision Where the lymphatics had been cut across they remained open for many hours, often for 24 to 48 hours, in contrast to the blood vessels, which constricted and closed almost at once. During this time any particles in the incision, such as red cells or bacteria, readily enter these lymphatics From 24 to 48 hours after making the incision the cut lymphatics became blocked probably by fibrinous plugs which could be dislodged by injecting the lymphatics of the ear under a fairly high pressure of 40 to 80 cm water. After this time the lymphatics began to regenerate, and 7 to 10 days after incising the skin, dye injection showed up a wealth of small channels in and about the incision and some reconstituted channels could be observed passing directly through the incised area, Fig 94.

McMaster and Hudack also observed the reactions of the lymph vessels of the mouse's ear in response to a thermal burn For 24 to 48 hours after a second or third degree burn, the localized burned patches remained ischaemic, surrounded by regions of hyperaemia and oedema. The lymphatics in this burned area were also blocked by fibrinous clots or by heat coagulation as no dye injected into the ear lymphatics entered the area. Later in the recovery stage, as with simple incisions, the lymphatics surrounding the burn showed considerable proliferation, Fig 95

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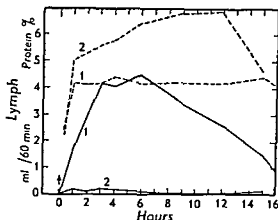


FIG 93 —*The effect on lymph flow and protein content of immersing the front feet of a dog in water at 100° C for 2 minutes at arrow* o Curves 1 and 1 for the right

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Regeneration of lymphatics in healing wounds. Complete restoration

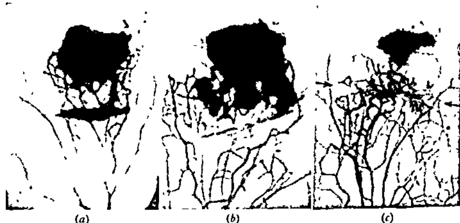
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In the healing of wounds, therefore, the regeneration of the lymphatic capillaries is just as prominent a feature as the growth of the blood vessels. No doubt the new lymphatics play an important rôle in removing protein and particulate matter from the injured tissue. In this way they



(a) (b) (c)

FIG 94

(From McMaster, 1942)



(a) (b) (c)

FIG 95

(From McMaster, 1942)

are essential in reducing the oedema in healing wounds. Always the regeneration of the lymphatics follows that of the blood vessels. Sometimes the delay between the proliferation of the blood capillaries and of the lymphatic capillaries may be considerable. For example, Clark and Clark (1932), using the rabbit ear-chamber preparation, showed that on the average blood capillaries invaded the table area 7 days after operation, while the lymphatic capillaries did so after 19 days. In some cases, however, the delay was much greater than this. In one case lymphatics did not grow into the table area until 40 days after the operation, whereas the blood capillaries did so after 5 days, and vascularization was complete 24 days before the first appearance of a new lymph capillary at the table edge.

The cause of this differential growth rate is not altogether clear. The growing blood capillary contains its own nutriment, whereas the lymphatics ... supply from nearby blood vessels.

... and Clark observed that lymphatic growth was ... tions in which fibrous tissue formation was excessive.

Whatever the cause of this disparity in growth rate between blood and lymphatic capillaries, it would appear reasonable to suppose that it can create a temporary *disturbance of the normal tissue fluid equilibrium*. For a varying period a tissue has blood capillaries but not lymphatic ones, with the result that a *local oedema* may develop. It is quite possible that this may be the basic factor responsible for the "hypertrophy" of scars which is so frequently found (Glucksman, 1951; Mowlem, 1951) and in which case as Mowlem and others have pointed out, *the surface* ...

... of interest in this connection that as far back as 1863 Billroth noted that during the first week of healing no lymphatic vessels were to be found in a young cicatrix. Although Reichert's (1926) experiments have shown that lymphatic regeneration across a wound may occur in 4 days, it is only in exceptionally favourable cases that this happens.

It is certainly the case that young scars tend to possess a characteristically oedematous appearance, as also does granulation tissue. However, there are a number of factors involved about which we are not altogether clear, and both species and regional differences may be concerned. Thus, in the inflamed ear of the mouse, Pullinger and Florey (1937) found that lymphatic pr... ...ly and fully. Furthermore, even where this ly... hypertrophic ...

filtrate is escaping from the blood capillaries all the time, but must percolate through the regenerating area until it reaches an adjoining region with a *normal complement of lymphatic capillaries* before it can be drained away by the lymph. As a rule equilibrium is restored by both the ingrowth of lymphatic vessels, and the gradual loss of vascularity, so that a scar becomes progressively paler. But should either or both of these changes not occur, the stage would seem to be set for much more extensive scar hypertrophy and even keloid formation (Yoffey, 1954).

Traumatic Shock

If the local loss of fluid following an injury is sufficiently rapid and extensive, a condition of irreversible peripheral circulatory failure or "shock" will develop (cf. Blalock, 1930; 1931*a*, *b* and *c*; Parsons and Phemister, 1930, Harkins, 1941). We have discussed the behaviour of the blood and lymphatic capillaries in the local injured region There has been for many years, and there still is, much controversy concerning the state and the permeability of the capillaries of the uninjured tissues in traumatic shock. At first, as fluid escapes from the blood stream into the injured area, tissue fluid is reabsorbed from the uninjured tissues and so compensates in some measure for the loss of blood volume The extent to which the blood volume falls depends upon the imbalance between these two fluid shifts In the early stages of an injury the inward shift of fluid from the uninjured tissues is slower than the outward passage of fluid into the injured area, so that if there is no red cell loss, the blood will become concentrated. When the loss of fluid in the injured region is speeded up by keeping it warm, the reabsorption of fluid from the uninjured tissues is also increased (Courtice, 1946). The rate of the inward shift of non-protein fluid through the blood capillaries, therefore, depends on the fall in capillary pressure which is determined by the rate and extent of fluid loss from the circulation in the injured area. The reabsorption of fluid also depends on the available extracellular fluid in the undamaged tissues If the animal has had a previously restricted water intake, the fall in blood volume is more rapid as reabsorption is less (Cameron and Courtice, 1946).

Most authors agree that in the onset of traumatic shock, the small blood vessels of the uninjured tissues are constricted and, in general, non-protein fluid passes from the tissue spaces into the blood stream. In the later stages of peripheral circulatory failure, however, much controversy has arisen concerning the state and permeability of the small blood vessels. Many toxic substances liberated from the damaged tissues have been postulated and more recently Zweifach and his colleagues have given evidence of a vasodepressant originating not in the damaged tissues but

in the liver (Shorr, Zweifach and Furchgott, 1945; Zweifach *et al*., 1945; Mazur and Shorr, 1948; Shorr *et al*., 1951).

We cannot here consider at any length the vascular changes of traumatic shock. We are mainly concerned, as far as the rôle of the lymphatics is concerned, with the efficiency of the capillaries of the undamaged tissues as barriers to the plasma proteins. This has been determined by two methods. By collecting lymph from uninjured areas, it has been shown that the extravascular circulation of protein in these regions has not been increased in animals suffering severe shock resulting from thermal or chemical burns (Glenn, Muus and Drinker, 1943; Cameron, Courtice and Short, 1947). In other experiments labelled plasma proteins have been injected intravenously in animals suffering from severe shock caused by haemorrhage, thermal burns or mechanical trauma (Fine and Seligman, 1943, 1944; Cope and Moore, 1944). These experiments suggest that the permeability of the capillary wall to protein is not greatly increased as it is in the injured tissues.

Lymphatic Obstruction

Acute lymphatic obstruction

The condition of acute lymphatic obstruction is readily produced in toads and in frogs either by destruction of the lymph hearts or by paralysis of the hearts with curare. Owing to the fact that the capillaries of the frog are highly permeable to water and to dissolved substances, including the plasma proteins, it is essential in these animals that extravascular fluid move rapidly back into the circulation. When this fails to occur, oedema develops, and the animal dies from loss of blood volume (see Chapter 4).

In mammals it is difficult to produce a complete blockage of the return of lymph to the blood stream because of accessory anastomotic channels. Ligature of the thoracic duct at the point of venous entrance may cause temporary lymph stasis and even chylous collections in the peritoneal, pleural and pericardial sacs, but ordinarily after a brief period of stasis lymph begins to enter the circulation through collateral venous connections (see p. 13, Chapter 1). Blalock, *et al* (1937) tried to produce complete lymphatic blockage in 52 dogs and 22 cats. Operations were carried out to block the lymph ducts in the neck and chest, to destroy the cisterna chyli and to interfere with the drainage of the mesenteric lymphatics. Assessed by the blood picture, temporary obstruction was obtained in many of the animals, but soon the blood picture returned to normal. In most of the animals in which evidence of blockage disappeared, lymphatic communications with the inferior vena cava were demonstrated at autopsy. Permanent complete blockage was, however, attained in 3 dogs

in whose blood the lymphocytes and eosinophils almost disappeared. The animals lost weight rapidly and were killed when it was obvious that they were going to die. At autopsy no lymphaticovenous communications were demonstrated.

We see, therefore, that although ligature or blockage of the thoracic duct will cause a temporary obstruction to the return of lymph, collateral channels will in most cases ultimately function efficiently in transporting the lymph to the blood stream. This is also true of any other main lymphatic channel which is ligated. Lymphatic obstruction may, however, occur for a period long enough to produce oedema. In the experiments of Reichert (p 18) in which all the soft tissues in the leg of a dog except the artery, vein and nerve were sectioned, transient lymphoedema occurred below the incision but disappeared when regenerating lymphatics bridged the gap between the incised tissues. Halsted (1921) attributed oedema of the arm after radical removal of the breast for carcinoma to the blockage of lymphatics by scar tissues. Eloesser (1923) made transverse incisions on the surface of the rabbit's ear and found that the subsequent scar blocked the flow of lymph. When India ink was injected slowly at the tip of the ear, it coursed along the lymphatics but stopped at the scar. On continuing the injection some ink passed slowly through constricted channels in the scar and then passed quickly along the vessels on the other side.

Chronic lymphatic obstruction

The changes observed in tissues which have been the subject of repeated attacks of inflammation or of long continued mild irritation are not separable from the conditions observed in chronic lymphatic obstruction Efforts to produce elephantiatic changes in animals by measures assumed to interrupt lymph drainage have a long history of ill success. Cohnheim (1889) believed oedema would not follow even total occlusion of the lymphatics to a part; but this was pure belief—not experimental fact. As Reichert (1926) has shown, section of the lymphatics blocks the lymph return temporarily and causes transient oedema. Regeneration of the lymphatics, however, made it impossible to produce lymph-oedema and elephantiasis, diseases which are common in certain parts of the tropics and not rare even in northern climates. Wherever such conditions are seen, they develop slowly; they are not the result of a single episode, such as the operation described by Reichert, but of repeated obstruction Recognizing this fact, Drinker and his colleagues made repeated injections of a suspension of quinine hydrochloride and crystalline silica into the lymphatics of the leg of a dog. The procedure adopted is illustrated in Fig. 96 (Drinker, Field and Homans, 1934). In the case illustrated, between December 12th and March 26th lymphatics were cannulated

centrally at the points shown; on each occasion from 3 to 5 ml. of the silica-quinine suspension were injected. After the first two or three injections a pitting oedema occurred, but was not sustained. Gradually the oedema became permanent and the leg began to feel brawny, indicating the beginning of fibrous or elephantiatic change. The injections continued until no more lymphatic trunks could be found for cannulation. The end result in such an experiment can be somewhat hastened by the surgical removal of the iliac lymph nodes and by intralymphatic injections of the opposite leg, but neither of these procedures is essential. The injections never caused pain or redness, and on recovering from the nembutal anaesthesia the dogs immediately ran about as usual and showed

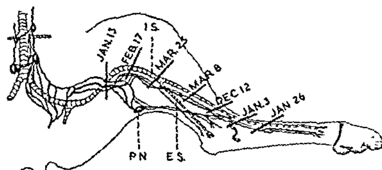


FIG. 96.—Lymphatics in the hind leg of the dog, with sites of successive central injections of quinine hydrochloride and crystalline silica indicated by bars. *P.N.*, popliteal node; *E.S.*, external saphenous vein; *I.S.*, internal saphenous vein. (From Drinker, Field and Homans, 1934.)

no signs of lameness. The condition eventually produced was a typical lymphoedema and a progressive elephantiasis, with extensive overgrowth of the subcutaneous connective tissue, and eventually with overgrowth of the epithelium in the foot pads.

Examination at autopsy of an elephantiatic area showed sclerosis of lymph nodes and large lymphatic trunks. Histologically the thickened skin and subcutaneous tissue showed marked fibrosis, the individual fibres being separated by oedema fluid. Here and there were found collections of lymphocytes and, particularly in the skin, many widely dilated lymphatics, lined by a single layer of endothelium. It was quite apparent that the lymphatic network in the skin, though possibly damaged, was not destroyed.

As oedema developed, the fluid could readily be obtained for analysis. The protein content soon rose above 2 per cent and in one animal it rose as high as 5 per cent. Table 55 presents a comparison between the chemical composition of plasma and oedema fluid from the legs of three

in whose blood the lymphocytes and eosinophils almost disappeared. The animals lost weight rapidly and were killed when it was obvious that they were going to die. At autopsy no lymphaticovenous communications were demonstrated.

We see, therefore, that although ligature or blockage of the thoracic duct will cause a temporary obstruction to the return of lymph, collateral channels will in most cases ultimately function efficiently in transporting the lymph to the blood stream. This is also true of any other main lymphatic channel which is ligated. Lymphatic obstruction may, however, occur for a period long enough to produce oedema. In the experiments of Reichert (p 18) in which all the soft tissues in the leg of a dog except the artery, vein and nerve were sectioned, transient lymphoedema occurred below the incision but disappeared when regenerating lymphatics bridged the gap between the incised tissues. Halsted (1921) attributed oedema of the arm after radical removal of the breast for carcinoma to the blockage of lymphatics by scar tissues. Eloesser (1923) made transverse incisions on the surface of the rabbit's ear and found that the subsequent scar blocked the flow of lymph. When India ink was injected slowly at the tip of the ear, it coursed along the lymphatics but stopped at the scar. On continuing the injection some ink passed slowly through constricted channels in the scar and then passed quickly along the vessels on the other side.

Chronic lymphatic obstruction

The changes observed in tissues which have been the subject of repeated attacks of inflammation or of long continued mild irritation are not separable from the conditions observed in chronic lymphatic obstruction. Efforts to produce elephantiatic changes in animals by measures assumed to interrupt lymph drainage have a long history of ill success. Cohnheim (1889) believed oedema would not follow even total occlusion of the lymphatics to a part; but this was pure belief—not experimental fact. As Reichert (1926) has shown, section of the lymphatics blocks the lymph return temporarily and causes transient oedema. Regeneration of the lymphatics, however, made it impossible to produce lymph-oedema and elephantiasis, diseases which are common in certain parts of the tropics and not rare even in northern climates. Wherever such conditions are seen, they develop slowly; they are not the result of a single episode, such as the operation described by Reichert, but of repeated obstruction. Recognizing this fact, Drinker and his colleagues made repeated injections of a suspension of quinine hydrochloride and crystalline silica into the lymphatics of the leg of a dog The procedure adopted is illustrated in Fig. 96 (Drinker, Field and Homans, 1934) In the case illustrated, between December 12th and March 26th lymphatics were cannulated

flowed laterally or in a retrograde direction, but Kinmonth could not demonstrate any localized obstruction. He suggests that there may be other factors in the disease such as alterations in the rate of capillary filtration. The protein concentration in the oedema fluid from 15 patients with primary lymphoedema varied from 0·76 to 5·0 g per cent with an average of 2·5 per cent which is about the same as that observed in experimental elephantiasis in the dog. When labelled plasma proteins

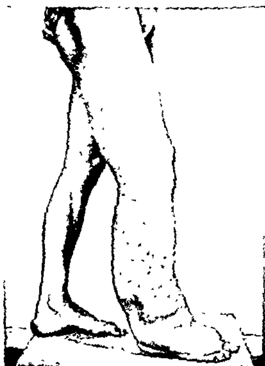


FIG. 97.—Unilateral lymphoedema and elephantiasis of the leg

A male, aged 18 years, with six years' history of steadily increasing oedema of the right leg. No known or discovered cause.

(By courtesy of Mr G. M. FitzGibbon, Frenchay Hospital, Bristol)

were injected subcutaneously into the lymphoedematous legs of two patients, their appearance in the blood stream was very slow—only about one-tenth the rate that occurs in normal legs.

A congenital condition of lymphoedema also occurs in Ayrshire calves (Hancock, 1950; Donald, Deas and Wilson, 1952). Morris *et al.* (1954) examined the lymphatic apparatus in two such cases. The limbs and head showed typical elephantiasis; the lymph vessels everywhere were extremely dilated, thickened and tortuous; and although the primary lymph

TABLE 55

Composition of plasma and oedema fluid from three dogs with chronic lymphatic obstruction

Date	Protein (g./100 ml.) Plasma	Protein (g./100 ml.) Oedema Fluid	Albumin (g./100 ml.) Plasma	Albumin (g./100 ml.) Oedema Fluid	Globulin (g./100 ml.) Plasma	Globulin (g./100 ml.) Oedema Fluid
Dog 1						
July 13, 1933	6·08	2·82	2·46	1·25	3·62	1·57
,, 13	6·08	2·25	2·46	0·98	3·62	1·27
,, 26 (1st day of attack)	6·42	3·75	2·85	1·64	3·57	2·11
,, 27 (2nd ,, ,, ,,)	6·88	4·08	2·90	1·75	3·98	2·33
Dec 20	7·3	4·4	—	—	—	—
Feb 7, 1934	8·13	4·11	2·82	1·76	4·31	2·35
,, 15	7·12	4·58	2·68	1·57	4·44	3·01
May 4	7·38	4·15	2·78	1·63	4·60	2·52
Dog 2						
July 29, 1933 (day after induced attack)	—	3·04	—	1·93	—	1·11
Feb 15, 1934	7·30	3·33	3·44	1·86	3·86	1·47
Apr 30	6·57	4·08	4·15	2·21	2·42	1·87
Dog 3						
June 30, 1933 (day of streptococci inj.)	6·99	3·85	3·24	2·15	2·74	1·70
July 1 (day of streptococci inj.)	6·73	3·53	3·11	1·97	3·62	1·56
Aug 9	—	3·48	—	2·07	—	1·41
Apr 6	7·11	2·71	4·24	1·77	2·87	0·94

From Drinker, Field and Homans (1934)

dogs with experimental lymphoedema and elephantiasis (Drinker *et al.*, 1934).

In man lymphoedema is relatively common in some tropical countries, the causal agent being *Filaria Bancrofti.* The micro-filariae are carried in the blood and lymphatic vessels and have a particular predilection for lymphatic channels and lymphatic glands which may become obstructed in any part of the body by the adult worms. Obstruction leads at first to oedema which pits on pressure, but in time extensive fibrous overgrowth may occur, Fig 97. These elephantiatic changes are usually seen in the legs, scrotum, penis and vulva.

In non-tropical countries lymphoedema may be secondary to malignant or inflammatory disease,
obscure. Kinmonth anc
lymphatics by injections
the intralymphatic injection of diodone in a group of patients with primary lymphoedema. Fig. 98(*a*) shows the radiographic picture of normal lymphatic channels in the leg while Fig. 98(*b*) shows the picture in a lymphoedematous leg. In the elephantiatic limbs, the lymph vessels were often enlarged and very tortuous and the valves incompetent, so that dye

lymphoedema become established the regions involved become peculiarly susceptible to infection, and so far as is known this is usually streptococcic. This was clearly shown in experimental lymphoedema. Dogs are relatively insusceptible to streptococcal infection, and the organisms isolated from attacks of infection in the lymphoedematous legs of experimental animals never caused more than the most transient local disturbance if injected into sound parts of the same animals or into normal dogs. Yet the lymphoedematous leg was not only susceptible to spontaneous streptococcal infection, but could be infected very readily by injection of streptococci isolated during previous seizures (Drinker *et al.*, 1935).

It is evident that when lymph drainage to a part is interrupted so that the various substances, which under normal and abnormal conditions are removed by the lymphatics remain *in situ*, two things happen. First of all, there is extensive fibrous overgrowth and even involvement of epithelium. Second, the part becomes strikingly susceptible to infection particularly to infection by streptococci Furthermore—and this is important in relating the functions of the lymphatic apparatus to the distressing development of elephantiasis—each attack of local infection simply intensifies what will occur inevitably, but more slowly, if lymph drainage is blocked but infection does not occur.

Inflammation is accompanied by increase in capillary permeability An attack of what has been called "elephantoid fever" in an affected part results in the profuse escape of blood plasma and of leucocytes into the region. The leucocytes, for the most part, seem to disintegrate, soon becoming shadowy, poorly stained members of oedema fluid films. Practically all of the abnormal constituents of the oedema fluid depend upon the lymphatics for removal, and if lymphatic obstruction is present, the result is highly unfavourable for the subject All that happens in

when infection exists.

when materials normally

rgrowth which inevitably

takes place is of what may be called normal type. It is the extensive sort of scar tissue formation seen about neglected and infected wounds. What one sees is a generalized scar-like formation, developing under epithelium which is at first normal, but which later is perhaps itself overgrown.

Lymphatic fistulae

Until recently, attempts to produce experimentally a lymphatic fistula which would flow freely for several days have not been successful. With the introduction of polyethylene or transflex tubing, however, in which the lymph does not readily clot, fistulae in rats and in dogs have flowed for times up to a week or more. Experimental thoracic duct fistulae have

nodes, i e. the nodes arising from the embryonic lymph sacs, were normal the secondary nodes were grossly abnormal. Dyed plasma injected into a limb did not pass very far along the grossly tortuous vessels. At post mortem no blockage could be found, but on cannulating the thoracic duct in the abdomen, it was found impossible to force fluid along it even at high



(a) (b)

FIG 98—The lymphatic channels of the leg shown by X-ray after the injection of diodone into lymphatics at the ankle

(a) Normal leg
(b) Lymphoedematous leg

(From Kinmonth, 1954)

pressure. On examination, no blockage was present but the gross tortuosities of the duct prevented the onward flow of lymph. It seems certain that in these cases as well as in the human cases described by Gager (1923), Mason and Allen (1935) or Kinmonth (1954) infection was not necessary to cause the development of elephantiasis; nor was infection necessary in the experimental elephantiasis of Drinker and his colleagues.

Of greater interest is the fact that when lymphatic obstruction and

the animals from injections of diphtheria and tetanus toxins and of tiger-snake venom into the leg, but not from injections of strychnine or of cobra venom which has a small molecule. These latter substances are readily absorbed into the blood stream, whereas the large protein molecules of the other toxins injected pass only into the lymphatics.

Relation of lymphatic system to blood infections

The lymphatic system, in general, readily becomes invaded by bacteria present in the blood stream. Drinker *et al.* (1935) infected rabbits by intravenous injection of cultures of a type III pneumococcus, strain SV. This organism is highly virulent for rabbits, causing death in 1 to 8 days, depending on the size of the dose After infection and under nembutal anaesthesia, thoracic duct lymph and blood specimens were collected and cultured for pneumococci. When large doses of pneumococci were given intravenously, organisms were found after 1 hour in the thoracic duct lymph. If animals were infected with small doses and lymph and blood examined after 20 hours, both fluids were invariably positive for pneumococci; and the lymph might contain more organisms per ml. than the blood. Organisms in the blood stream can therefore escape through the capillaries and infect the tissue fluid and lymph

The effect of intravenously administered specific antiserum on the organisms in the blood will depend on the dose given The concentration of antisera in the tissue fluid and lymph, however, will depend on the permeability of the capillaries to the globulins and will be much less in lymph than in the blood stream Drinker and his colleagues showed the effect of antipneumococcal serum on the blood and lymph of rabbits after the intravenous injection of pneumococci In Fig. 99*a* a rabbit, 20 hours after intravenous injection of pneumococci, showed 10^4 colonies of organisms per ml of blood and 10^3 colonies per ml. of thoracic duct lymph. After two and a half hours, during which the organisms increased in both fluids, 8 ml. of antipneumococcus type III serum were given intravenously The blood promptly became almost sterile, but the count of organisms in the lymph was little reduced. Fig. 99*b* shows a second experiment in which the number of organisms in the thoracic duct lymph was greater than in the blood. Here again antiserum sterilized the blood, but the lymph was unaffected. It is evident, therefore, that when a large dose of antiserum is given intravenously, it may reach sterilizing concentrations in the blood, but fail to do so in the lymph, with the result that the lymphatic system becomes a "hideout" for infecting organisms, which after a time may move on into the blood and re-establish blood infection. Anyone who has seen human streptococcal infections treated with antisera is unhappily familiar with just this chain of events. Sulphonamides and antibiotics, on the other hand, readily diffuse through the blood capillaries

with obstructed lymphatics survived much longer than similarly injected control animals. It is reasonable to believe, therefore, that in the early stages of a localized bacterial infection, some of the bacteria or of their toxins will be drained away by the lymphatics. The involvement of these vessels may be an important line of defence, because the lymph passes through a lymph node or nodes before entering the blood stream, and, as far as bacteria are concerned, lymph nodes are on occasion very effective filters (Drinker, Field and Ward, 1934), as well as being the site of a certain amount of anti-body formation (see Chapter 5).

These principles apply not only in the more common pyogenic infections of the skin, but also in other bacterial diseases. For example, the involvement of the local lymph nodes in tuberculosis follows the entrance of organisms either through the respiratory or alimentary tracts. Experimentally, the intradermal inoculation of the tubercle bacillus resulted in the involvement of the regional nodes as early as 24 to 48 hours later (Freund and Angevine, 1938).

The function of the lymph nodes in holding up and destroying bacteria by phagocytic action of polymorphonuclear leucocytes and macrophages has been described in Chapter 5. Viruses, too, travel from the portal of entry along the lymphatics to the nearest lymph node, where instead of being ingested they may multiply (see Chapters 3, 5, and 7)

It may only be necessary for a small amount of toxic material to pass along the lymphatic vessels to set up lymphangitis or involve the regional lymph nodes However, the larger this amount, the more likely some of it will pass through the node and enter the blood stream to give rise to a bacteraemia. The actual lymph flow may, therefore, be important in the spread of infection We know that when a lymph duct is cannulated in the leg of an animal, passive or active movement very greatly increases the volume of lymph collected. In man, too, McMaster has shown that the flow of lymph in the skin lymphatics is greatly increased by movement, more especially in any inflammatory condition where oedema is present. Not only is the spread of infective material from the local lesion greatly increased by movement, but the lymph nodes become less efficient as filters when the flow and pressure of lymph increase Field, Drinker and Ward showed this experimentally in the perfusion of a lymph node with a culture of streptococci.

The importance of rest in acute bacterial infection has long been advocated and more recently stressed by Trueta (1946) in his treatment of infected wounds by complete immobilization The transport of toxic products from an infected limb should be mainly by the lymphatics and this should be minimal when the limb is immobilized in plaster. To test this experimentally, Barnes and Trueta (1941) completely immobilized the hind limbs of rabbits in a plaster cast and found that this protected

the passage of such an embolus along a lymphatic is extremely rapid, even though the volume of lymph flow may be very small. This is especially so if there is movement of the affected part.

For a detailed and authoritative account of the rôle of lymphatics in the spread of the different types of malignant tumour, Willis's book "The spread of tumours in the human body" (1952) should be consulted.

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into the tissue fluid so that the concentration in the lymph soon reaches values similar to those in the blood (cf. Hawking and Lawrence, 1950; Florey *et al.*, 1949). The effect of these more readily diffusable substances on the infecting organisms in the tissue fluid and lymph is, therefore, rapid.

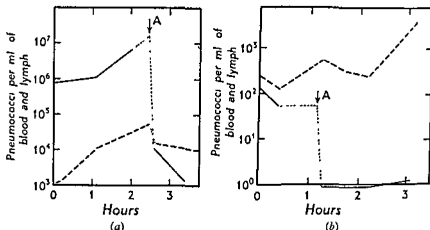


FIG 99

(*a*) Effect of antipneumococcus type III rabbit serum on the organisms in the blood and lymph of a rabbit 23 hours after infection ————, number of organisms in blood, – – – – –, number of organisms in lymph, ·········, probable number of pneumococci in blood and in lymph before and at the time of antiserum injection At A, 8 ml of antipneumococcus type III rabbit serum were given intravenously

(*b*) As for (*a*) At A, 10 ml of antipneumococcus type III horse serum were given intravenously

(From Drinker, Enders, Shaffer and Leigh, 1935)

LYMPHATIC SPREAD OF TUMOURS

Clinically, one of the most prominent features of the lymphatic system is its rôle in the spread of tumours. Malignant cells may extend into and occupy any available crevices in the invaded tissues. The lymphatics serve as one route by which many carcinomata may directly extend in this way. In this lymphatic permeation by malignant cells, the affected area presents a network of white lines of growth which clearly mark out the pattern of the lymphatic plexus. Columns of invading cells may be seen occupying distended lymphatics whose endothelial walls are still intact.

More prominent than the direct lymphatic spread of tumours is the great tendency of carcinomata to produce secondary growths in regional lymph glands Most of these metastases are due to detached tumour emboli which are carried to the glands in the lymph Once in the lymph stream the tumour emboli are not arrested until the lymph nodes are reached, since no tumour cells are observed intermediate between the primary carcinoma and the regional nodes. As we have seen in Chapter 4,

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